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TITLE:

Physiological Anatomical Rodent
Experiment (PARE) .04 Feasibility
Test 1

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Abstract. The objective this feasibility study was to investigate the environmental/treatment stresses in the proposed PARE.04 experiments in a ground based study to determine if these stresses were of sufficient magnitude to compromise the planned shuttle experiments. Eighty pregnant Sprague-Dawley rats were received on day 2 (day 1=day of vaginal plug) of gestation (G2) and on G7 60 were laparotomized to determine the condition of pregnancy and allow assignment to test groups. The five test groups (N=10 each group) were as follows: Group 1, nominal flight; Group 2, laparotomy control; Group 3, hysterectomy control; Group 4, vivarium control; Group 5, cesarean delivery. On G17, groups 1,2, and 5 were subjected to unilateral hysterectomy to obtain fetuses for evaluation. There was no difference in fetal crown-rump length, fetal weight, or placental weight in any of the test groups subjected to unilateral hysterectomy at G17. Animals were allowed to go to term and animals in each group delivered between the morning of G22 and the afternoon of G23. Rats assigned to Group 5 began delivering vaginally prior to the designated time for cesarean section, thus only 2 animals in this group were delivered by cesarean section. After delivery, a blood sample was taken from the dam, and they were euthanized and the thymus and adrenal glands weighed. Pups from experimental dams were tattooed for identification, the anogenital distance of male pups was photographed for later measurement, and all pups placed with foster dams and litter sizes were standardized to 10. On day 7, all pups were euthanized, and pup adrenal glands and thymus weighed. Laparotomy at G7 with or without unilateral hysterectomy at G17, had no effect on pregnancy maintenance or vaginal delivery. There was no difference in maternal adrenal or thymus weights or plasma levels of catecholamines, estradiol, progesterone or corticosterone. Likewise, there was no difference in the anogenital distance (index of fetal stress) of neonatal male pups, between any of the experimental groups. From days 0-7, weight gain from dams in all experimental groups was similar. Lastly, there was no difference in weights of thymus and adrenal glands in pups euthanized at day 7. Collectively, these data indicate that treatment stresses inherent in the proposed PARE .04 experimental design should not compromise the planned shuttle experiments.

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Introduction

The Physiological Anatomical Rodent Experiment (PARE) .04 is a shuttle payload expected to fly in September, 1994. The PARE .04 payload will be devoted to developmental biology experiments and will be the first US payload to fly pregnant rats. The experimental design for the PARE .04 studies was formulated to acquire the maximum scientific yield; however, it requires that pregnant rats be subjected to a series of environmental/treatment stresses.

The general objective of the present study (PARE .04 Feasibility Test 1) was to investigate the environmental/treatment stresses in the proposed PARE .04 experiments in a ground based study to determine if these stresses were of sufficient magnitude to compromise the feasibility of the planned shuttle experiments. Specific objectives of the present study included: (1) To determine the pregnancy success rate for the vendor (Taconic) supplied timed-pregnant rats and evaluate the effects of shipment stress on pregnancy viability, (2) To determine whether two surgical interventions within the peritoneal cavity, viz., laparotomy on gestation day 7 and unilateral hysterectomy on gestation day 17, would interfere with the maintenance of pregnancy and the occurrence of vaginal delivery, (3) To determine whether the experimental design evoked stress responses evidenced in neonatal pups and in hormonal levels in maternal plasma, and (4) To accumulate a body of normative data for reference purposes.

All studies contained in this report were reviewed and approved by the East Carolina University Animal Care and Use

Committee at its meeting on June 22, 1993.

Methods and Materials

Animals: General. Eighty nulliparous pregnant Sprague-Dawley rats (Taconic Farms, Germantown, NY) were received at ECU on gestation (G) day 2 of pregnancy and five experimental groups were selected from this pool. Sixty one rats at G19 or G20 were also received and served as foster dams. In all cases, gestation day 1 = day of vaginal plug or sperm. The animals were trucked from the vendor to the Albany, NY airport and shipped air freight (American Airlines) to the Raleigh-Durham, NC airport. Rats were picked up from the American freight terminal by ECU personnel and transported in an air-conditioned station wagon or van to Greenville, NC. Outside temperatures in eastern NC ranged from 90-95 degrees F on days of shipment and receipt of rats.

Animals were housed at ECU in a room with controlled lighting (lights on 06:00-18:00) and provided laboratory chow and NASA supplied food bars (or food bars alone) and water ad libitum. The rat cages were clear polycarbonate, approximately 19 inches long, 10 inches wide and 8 inches high, and the bedding was ground corncob (Bed-O-Cobs, Anderson, Maumee, Ohio).

Animals: Experimental Groups. Eighty rats received on G2 were housed singly upon receipt. On days G2-G8, between 06:00 and 09:00 hours, body weights, and consumption of food (food bar and chow) and water were recorded. On G7, rats were subjected to laparotomy to confirm pregnancy and determine the number of decidual swellings per uterine horn. The intent was to ensure

that rats assigned to groups 1, 3, and 5 had at least five decidual swellings per uterine horn. Twenty-nine of 60 rats (48%) laparotomized at G7 met these criteria. Thus it was necessary to assign some rats to Group 5 that had one uterine horn with only four decidual swellings. Rats were randomly assigned to groups 2 and 4 without laparotomy as per the experimental design. The five test groups (N=10 each group) as follows:

Group 1, Nominal Flight (received laparotomy G7 and unilateral hysterectomy G17, natural delivery)

Group 2, Laparotomy Control (received unilateral hysterectomy G17, natural delivery)

Group 3, Hysterectomy Control (received laparotomy G7, natural delivery)

Group 4, Vivarium Control (no laparotomy or hysterectomy, natural delivery)

Group 5, Cesarean Delivery (received laparotomy G7 and unilateral hysterectomy G17, planned cesarean delivery)

Animals: Surgery. For laparotomy at G7, rats were anesthetized by placing the animal's nose under a cone containing absorbent cotton damp with isoflurane (AErrane, Anaquest, Madison, WI). Hair was subsequently clipped, ophthalmic ointment (Ocutricin, Vedco, St. Joseph, MO) applied to the cornea, and the abdomen was cleaned with 70% ethyl alcohol. Anesthesia for laparotomy was maintained with isoflurane via a nose cone. The ventral skin, muscle wall, and parietal peritoneum were cut

approximately 2 cm cranial to the pubis and the incision extended cranially 2-3 cm. Each uterine horn was exposed and lifted with forceps at the tubal end and the decidual swellings counted and recorded. Subsequently, the peritoneum and muscle layer were sutured (interrupted sutures) with monofilament nylon (3-0 Ethilon, Ethicon, Somerville, NJ), the skin closed with 9 mm wound clips (Autoclip, Clay Adams, Parsippany, NJ), and the animals allowed to recover. Each laparotomy took approximately 10 minutes. Based on the number of decidual swellings recorded, thirty of the animals were selected for assignment to groups 1, 3, and 5 (N = 10 each group).

On G8, body weights of all animals were recorded and rats subjected to laparotomy closely examined. Wound clips on 8 of the 60 rats that had experienced surgery the previous day were found to be loose and were removed and replaced. Animals in all test groups were placed 5 rats per cage on G8 and provided NASA food bars and water ad libitum through G16. During the interval between G8 and G16, the animals were left undisturbed except for daily addition of food bar and water as required.

On G17, rats in Groups 1,2, and 5 were subjected to unilateral hysterectomy. For this procedure, animals were anesthetized and prepared for surgery as previously described for the laparotomy at G7. Wound clips were removed from animals in Groups 1 and 5. Prior to surgery, animals were injected sc with 10,000 units of Durapen (Vedco, Inc., Overland Park KS) and 5mg/kg of Butorphanol (Aveco Inc., Fort Dodge, IO). Sterile technique was employed and

instruments used in each surgical procedure were soaked at least 15 minutes in a germicide solution (Cetylcode, Pennsauken, NJ). The number of live fetuses in each horn was recorded. The uterine horn to be removed was alternated between rats in each of the three treatment groups. The horn was ligated cranially and caudally with black braided silk (2-0, Ethicon, Sommerville, NJ), and excised. The incision was closed with interrupted sutures using monofilament nylon (3-0 Ethilon, Ethicon, Somerville, NJ) and wound clips (9 mm autoclips, Clay Adams, Parsippany, NJ). Fetuses and placentae were quickly removed from the uterine horn, weighed, and weights recorded. The crown-rump length of each fetus was also recorded. The unilaterally hysterectomized rats were allowed to recover, returned to the animal room and singly caged.

One rat assigned to Group 2 was not pregnant at surgery on G17, however, this animal was still subjected to unilateral hysterectomy and otherwise treated identically to the other rats in Group 2.

On days G17 - G21, body weights, food bar consumed, and water consumed, were recorded daily for each rat in each of the five test groups.

Animals assigned to test groups started delivering naturally on the morning of G22, and it became apparent that if rats in the CD group were allowed to go to 15:00 hours on G23 before cesarean delivery, all would have delivered naturally. Therefore, two animals in group 5 were delivered by cesarean section between 06:00 and 07:00 hours on G23.

Animals: Cesarean Delivery. One dam was delivered by cesarean section under isoflurane anesthesia and one was delivered after cervical dislocation. To produce cervical location, a rod was pressed to the base of the skull. In both instances, the abdomen was opened, the uterine horn externalized, and incised. Pups were quickly removed from the uterus and placed on a saline soaked towel under a heat lamp. Membranes were removed and each pup stroked repeatedly with a foam brush to stimulate breathing. The umbilical cord was occluded with a battery powered electro-cautery.

Animals: Cross Fostering. Starting on day G22, rats designated to serve as foster dams and rats assigned to test groups were observed for the initiation of delivery at hourly intervals from 06:00 hours to 18:00 hours. Animals were not disturbed between 18:00 hours, day 22 and 06:00 hours, day 23. Thus the exact time of initiation/duration of delivery of foster and test dams with young in the cage at 06:00 hours on day 23 was not known. During the light phase of G23, animals were again observed at hourly intervals beginning at 06:00 hours. Once test animals had completed delivery, the number and sex of pups was recorded and each pup was numbered with a red or green tattoo for identification. All tattoos were applied on the dorsum of rat pups with a Spaulding Special Electric Tattoo Marker, Model SSEMK110 (Spaulding & Rogers, Voorheesville, NY). Subsequently, red or green (experimental) numbered pups were placed with a foster dam. In all cases, the foster dam had been nursing her own pups at least one

day prior to receiving the experimental pups. Pups delivered by the foster dams were numbered with a black tattoo for identification. The total litter number (natural pups plus foster pups) of all foster dams was adjusted to 10. In experimental groups 1,2, and 5, which had unilateral hysterectomy on day 17, and therefore delivered young from only one horn, all pups delivered were cross fostered. Rats in experimental groups 3 and 4 retained both uterine horns and usually delivered more than 10 pups. In these groups, all male pups and female pups necessary to total 9, were placed with foster dams. The foster dam was always allowed to retain at least 1 of her natural pups.

On the day pups were delivered, prior to cross fostering, the anogenital distance in all male pups, in all experimental groups was photographed. The anogenital distance is defined as the length of tissue separating the anus and genital papilla. This distance is an index of sexual differentiation and in male pups experiencing prenatal stress, this distance is decreased transiently in the postnatal period. The anogenital distance was photographed with an Olympus OM-1 camera attached to an Olympus Operation Microscope (Model MTX, Olympus Optical Co., Tokyo, Japan) equipped with automatic flash. The film used was Kodak T-Max black and white, ASA 100, and the magnification on the film was 2X. Black and white prints were enlarged to a total magnification of 16X, and the anogenital distance was measured from the prints with a 6 inch dial caliper and subsequently calculated and recorded.

While pups from experimental dams were being tattooed and

having photomicrographs made of the anogenital distance, experimental dams were anesthetized with isoflurane, the abdominal cavity opened, and the aorta exposed. At the aortic bifurcation, 6-8 cc of blood was drawn into a heparinized 10 cc syringe fitted with a 21 gauge needle. If animals did not die from exsanguination, they were euthanized with an overdose of isoflurane. The plasma was separated in a refrigerated centrifuge, decanted, and stored in a cryovial at -70 degrees C. The thymus and adrenal glands were removed, connective tissue was carefully trimmed, and the organ weights recorded.

Frozen plasma was shipped in dry ice via Federal Express to Nichols Institute (Los Angeles, CA) for determination of plasma concentration of progesterone, estradiol, corticosterone, and catecholamines (dopamine, norepinephrine, epinephrine).

Animals: Pups. The body weight of pups from test dams was recorded when they were placed with foster dams (day 0) and subsequently on neonatal days 2 through 7. At daily weighings, the red, green or black tattoos on the pups' back were enhanced as necessary with a red, green, or black "Sharpie" pen. On day 7, the pups were anesthetized with ether, decapitated, and the thymus and adrenal glands removed, cleaned, and weighed.

Statistics. Numerical data were expressed as mean +/- standard error of mean. Data in different groups were examined with one-way analysis of variance (ANOVA) and if significance was found with ANOVA, group comparisons of means were made with the Newman-Keuls test. The Student's t test was used to analyze data

between two samples.

Results

Animals: General. Taconic Farm pregnant sprague dawley rats proved to be a sturdy, yet docile strain of rat for these studies. The rats appeared relaxed and content when housed individually. When group housed, even after surgery, the pregnant rats were biocompatible, and were usually observed during the light phase of the cycle closely huddled together.

Animals: Weight Gain G2-G7. Eighty animals received at G2 of pregnancy weighed approximately 185g and gained up to 220g by G8 (Figs. 1-7). Laparotomy at day 7 appeared to slightly decrease body weight at G8 but this decrease was not significant (Figs. 2,3,5,7). The body weights of thirty animals laparotomized at G7 and subsequently culled from the study, were followed through G9 (Fig. 2). Again, there was no significant difference in body weights during days G7-G9 and by G9 animals were starting to regain weight.

Animals: Food and Water Consumed G2-G7. During G2-G7, rats were allowed free choice of lab chow or food bars and water ad libitum. When both foods were made available, rats consumed more lab chow in preference to food bars (compare Figs. 8-13 with Figs. 14-19). Laparotomy at G7 significantly ($p < 0.05$) reduced lab chow consumption (Figs. 8,9,11,13) at G7 but had no effect on food bar consumption (Figs. 14-19).

Water consumption by rats on days G2 through G7 is shown in Figs. 20-25. Laparotomy at G7 significantly ($p < 0.05$) reduced

water consumption at G7 (Figs. 20,21,23,25).

Animals: Day 7 Laparotomy. The number of decidual swellings in the left and right uterine horns in the laparotomized test groups is shown in Fig. 26. There appears to be an increased number of decidual swellings in the right horn in all three groups, however, this difference is statistically significant ($p < 0.05$) only in the nominal flight group.

Animals: Day 17 Unilateral Hysterectomy. The number of live fetuses in the left and right uterine horns in test groups unilaterally hysterectomized at day 17 is shown in Fig. 27. There appears to be an increased number of live fetuses in the right horn in all three groups, however, in each group, this increase is not statistically significant ($p > 0.05$).

Group 1 (nominal flight) and Group 2 (CD group) were both subjected to laparotomy at G7 and unilateral hysterectomy at G17. Since the number of decidual swellings in each horn was recorded at G7, and the number of live fetuses recorded at G17, conceptus wastage during this interval of pregnancy could be ascertained. There was no significant difference in the number of decidual swellings at G7 and the number of live fetuses in the same animal at G17 (Fig. 28). Thus, laparotomy at G7 does not affect subsequent fetal development up to G17.

There was no difference in fetal crown-rump length, fetal weight, or placental weight in any of the test groups subjected to unilateral hysterectomy at G-17 (Figs. 29-31).

Animals: Weight Gain G17-G22. Animals in all groups at G17

weighed approximately 250-270 grams (Fig. 32). Rats in three experimental groups (Nominal flight, laparotomy control, and CD group) were unilaterally hysterectomized at G17. Body weights were recorded daily for all ten animals in all five test groups during days G17-G21 (Fig. 32). Unilateral hysterectomy at G17 significantly reduced ($p < 0.05$) weight gain of pregnant rats in all three experimental groups during the period of G19 through G22 (Fig. 32).

Animals: Food and Water Consumed G17-G22. Unilateral hysterectomy at G17 in Test Groups 1,2, and 5 reduced significantly ($P < 0.05$) food bar consumption on days G17, G18, and G19 (Fig. 33). However, by G21, all groups were consuming an equal quantity of food.

Unilateral hysterectomy at G17 in Test Groups 1,2, and 5 did not significantly alter water consumption at G17 and succeeding days apparently because of the very high variance between groups.

Animals: Initiation of Delivery. Parturition was initiated in animals in all test groups between the morning of G22 and the afternoon of G23 (Fig. 35). In animals observed hourly during the light phase of the cycle, the duration of delivery ranged from 1 - 3 hours, with most animals completing delivery in approximately 2 hours. When it became apparent that animals in the CD Group would likely deliver vaginally before 15:00 hours on G23, two animals in this group were delivered by cesarean section.

Parturition was also initiated in all foster dams between the

morning of G22 and afternoon of G23 (Fig. 36).

The number of live fetuses remaining in utero at day 17, after unilateral hysterectomy, was recorded in Nominal Flight, Laparotomy Control, and the CD Groups and thus the number of young to be delivered could be anticipated in these animals. Figure 37 shows that there is no difference in the number of live fetuses recorded at G17 and the number of live pups delivered in these same animals. Thus, unilateral hysterectomy at G17 does not appear to affect the subsequent delivery of live pups from the remaining horn.

Organ Weights and Plasma Hormone Levels of Test Dams. There was no significant difference in combined adrenal weights (Fig. 38) or thymus weights (Fig. 39) between dams in any of the test groups. Similarly, there was no significant difference in plasma levels of progesterone, estradiol, corticosterone, and catecholamines (dopamine, norepinephrine, epinephrine) between dams in any of the test groups (Figs. 40-46). Values for thymus and adrenal weights and hormone levels for the non pregnant rat in Group 2 were included with the nine pregnant rats in Group 2 since retrospective statistics showed the inclusion of these values from this one animal would not alter results of the statistical analysis.

Anogenital Distance of Male Pups From Test Dams on Day of Delivery. There was no significant difference in the anogenital distance in pups from dams in any of the test groups (Fig. 47).

Body Weight of Test Pups Neonatal Days 0-7. Pups born to dams in test groups were placed with foster dams after the test dam was

euthanized. There was no significant difference in the body weight of rat pups derived from dams in any of the test groups during neonatal days 0 (day of birth) through 7 (Fig. 48).

Pup Organ Weights at Neonatal Day 7. There was no difference in the weight of the thymus (Fig. 49) or adrenal glands (Fig. 50) in pups from dams in any of the test groups.

Discussion and Conclusions

Animals: General. Taconic Farms outbred sprague dawley rats were shown to be an excellent strain of rat for these studies. The high ambient temperature and other shipping stresses did not appear to adversely affect the rats. There was a high pregnancy rate in rats shipped on G2: Only 2 of 80 rats received were subsequently shown to not be pregnant. All 60 rats shipped at G19 or G20 for use as foster dams were pregnant. Rats appeared content singly housed and were biocompatible when group housed, whether or not they had experienced surgery.

When the rats had free access to both laboratory chow and food bars, they ate more laboratory chow. However, if the food bar was the only food source, it was readily consumed.

Animals: Surgery. Isoflurane was shown to be an excellent anesthetic with a rapid onset of effect and a quick recovery. Although a nose cone was used to administer the drug in these studies, an anesthesia machine complete with vaporizer and scavenger system would be preferable and would utilize much less of this expensive drug. Nonetheless, in the present studies, no animals were lost in any of the laparotomy or unilateral

hysterectomy procedures using a nose cone.

Laparotomy at G7 transiently affects food and water consumption and thus weight gain, however there is no evidence that this procedure stresses the animals or alters subsequent fetal development. Laparotomy at G7 showed that only 48% of 60 pregnant rats had at least 5 decidual swellings in each uterine horn. Thus the requirement that each uterine horn contain at least 5 decidual swellings in order to be assigned to a test group appears to be too stringent. The present results indicate that a selection criterion of 4 decidual swellings per uterine horn would be more plausible and allow the use of fewer rats.

Unilateral hysterectomy at G17, like laparotomy at G7, transiently depresses food consumption and thus weight gain. Unilateral hysterectomy did not alter subsequent fetal development in the remaining horn in any experimental animals. There were no differences in 20 animals that experienced laparotomy at G7 and unilateral hysterectomy at G17. These studies demonstrated that two successive surgical procedures into the peritoneal cavity do not affect pregnancy.

Laparotomy at G7, or unilateral hysterectomy at G17, did not alter normal vaginal delivery. Also, in twenty animals subjected to both procedures, there was no effect on the initiation or completion of vaginal delivery. Since surgery during pregnancy did not affect delivery, there was no reason to deliver any pups by cesarean section, other than to validate the technique.

Analysis of dam hormone levels, organ weights, and male pup

anogenital distances, corroborate that the surgical procedures did not adversely affect the dam or stress the pups in utero.

Pup Tattoo. The technique of applying different colored tattoos on the dorsum of neonatal rat pups that were to be cross fostered was validated. Body weight of individual pups could subsequently be charted. In all cases, foster dams appeared to readily accept and nurse experimental pups.

General Conclusion. These studies confirmed the feasibility, under conditions of gravity, of the proposed experimental design for PARE. 04. The docile behavior and biocompatibility under various experimental conditions of Taconic Farms sprague dawley rats was substantiated. It was demonstrated that two successive surgeries into the peritoneal cavity (laparotomy at G7 and unilateral hysterectomy at G17) did not alter fetal development, the duration of pregnancy, vaginal delivery, or maternal and fetal stress responses. It was also affirmed that the cross fostering technique is a valid, reliable procedure for rearing neonatal pups.

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**FIG.1 BODY WEIGHT (Mean \pm SEM) OF ALL ANIMALS
RECEIVED AT G2 n=80**

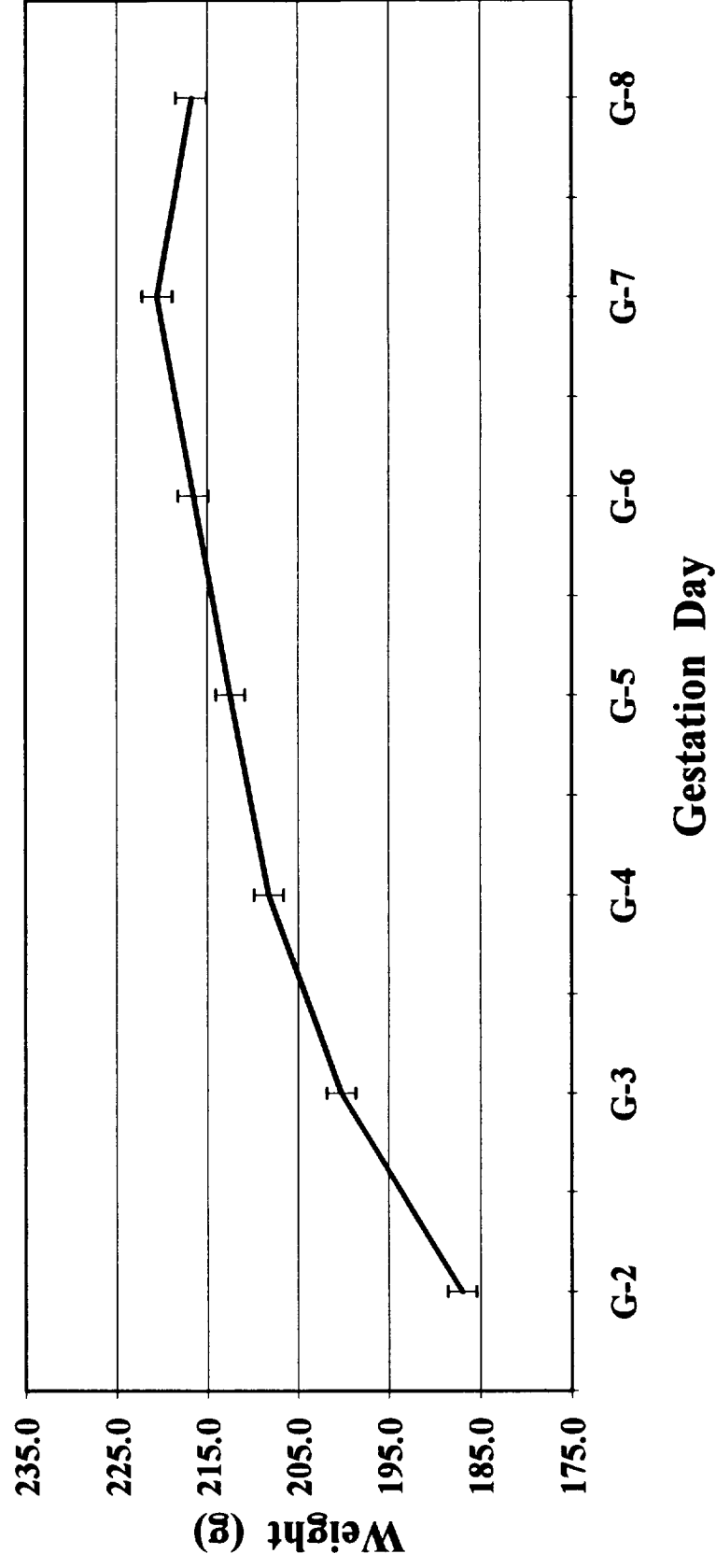


FIG.2 BODY WEIGHT (Mean +/- SEM) OF ANIMALS
CULLED n=30

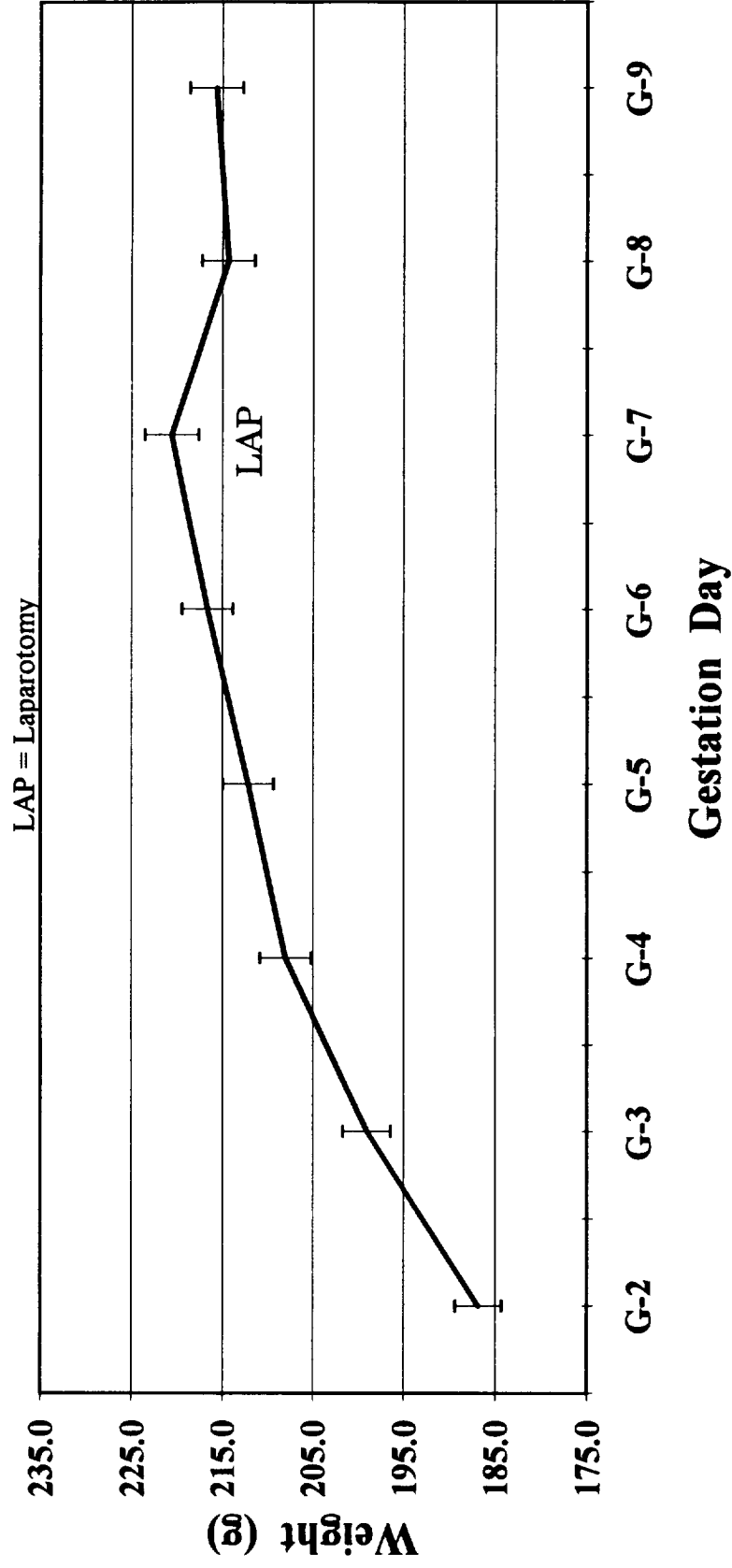


FIG.3 BODY WEIGHT (Mean +/- SEM) OF GROUP 1
(NOMINAL FLIGHT) ANIMALS n=10

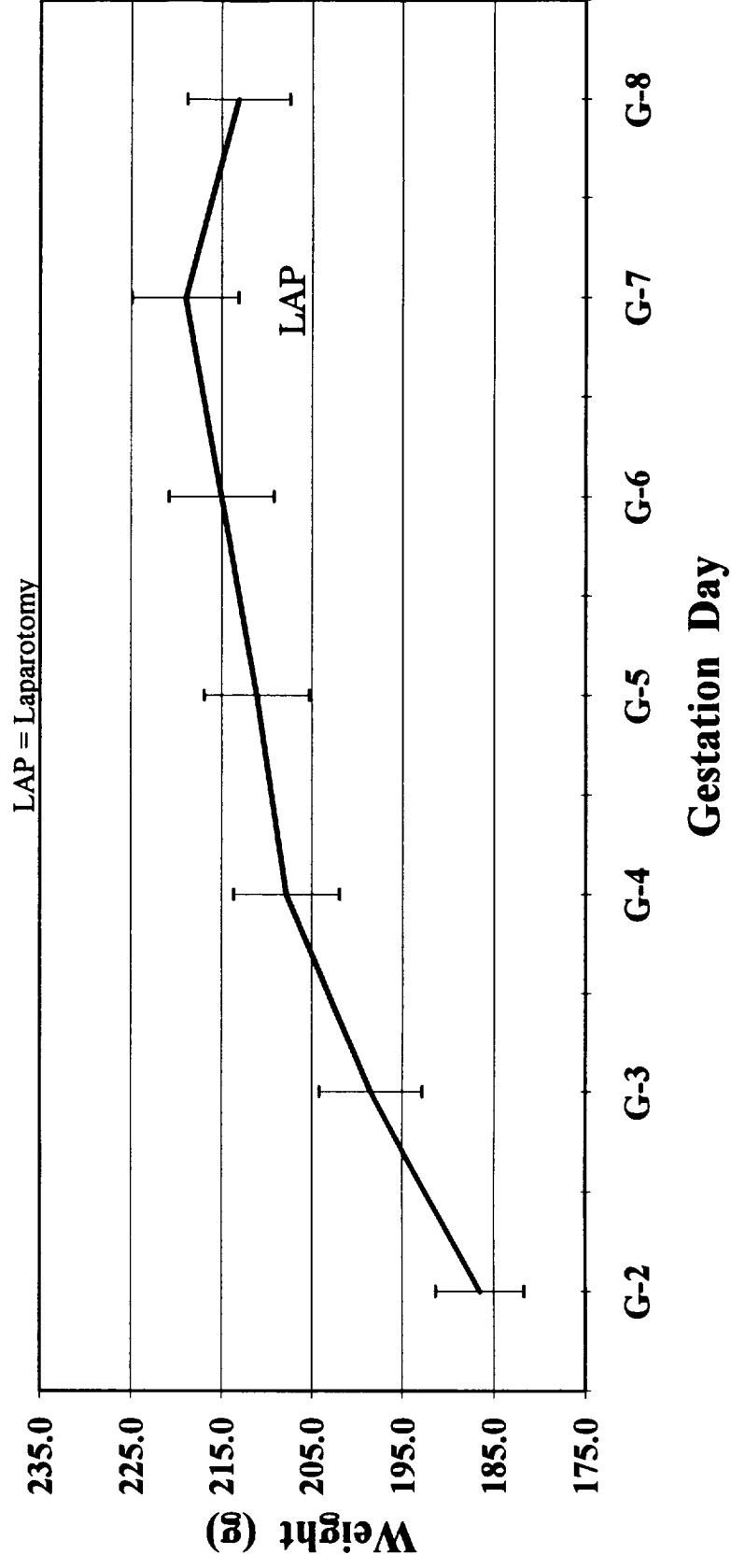


FIG.4 BODY WEIGHT (Mean +/- SEM) OF GROUP 2
(LAPAROTOMY CONTROL) ANIMALS n=10

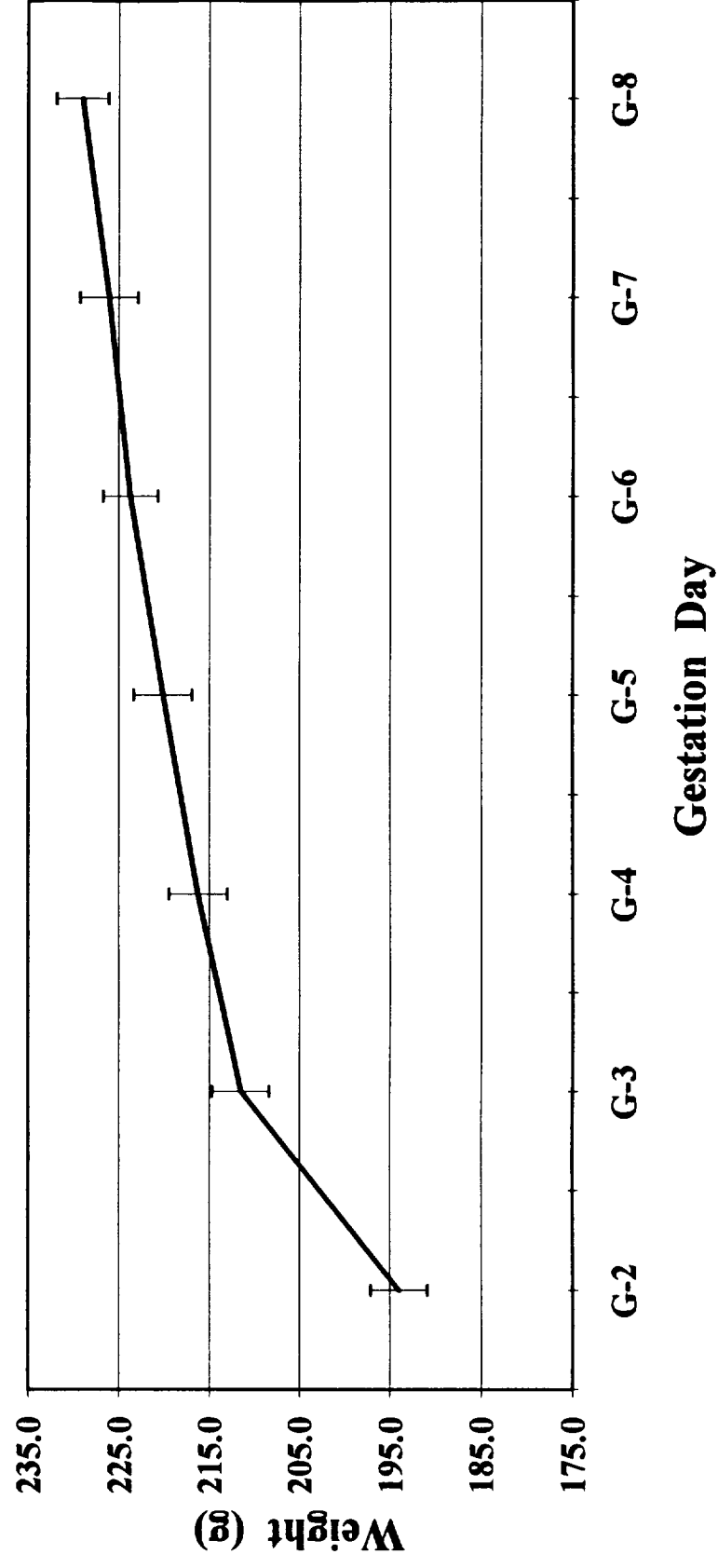


FIG.5 BODY WEIGHT (Mean +/- SEM) OF GROUP 3
(HYSTERECTOMY CONTROL) ANIMALS n=10

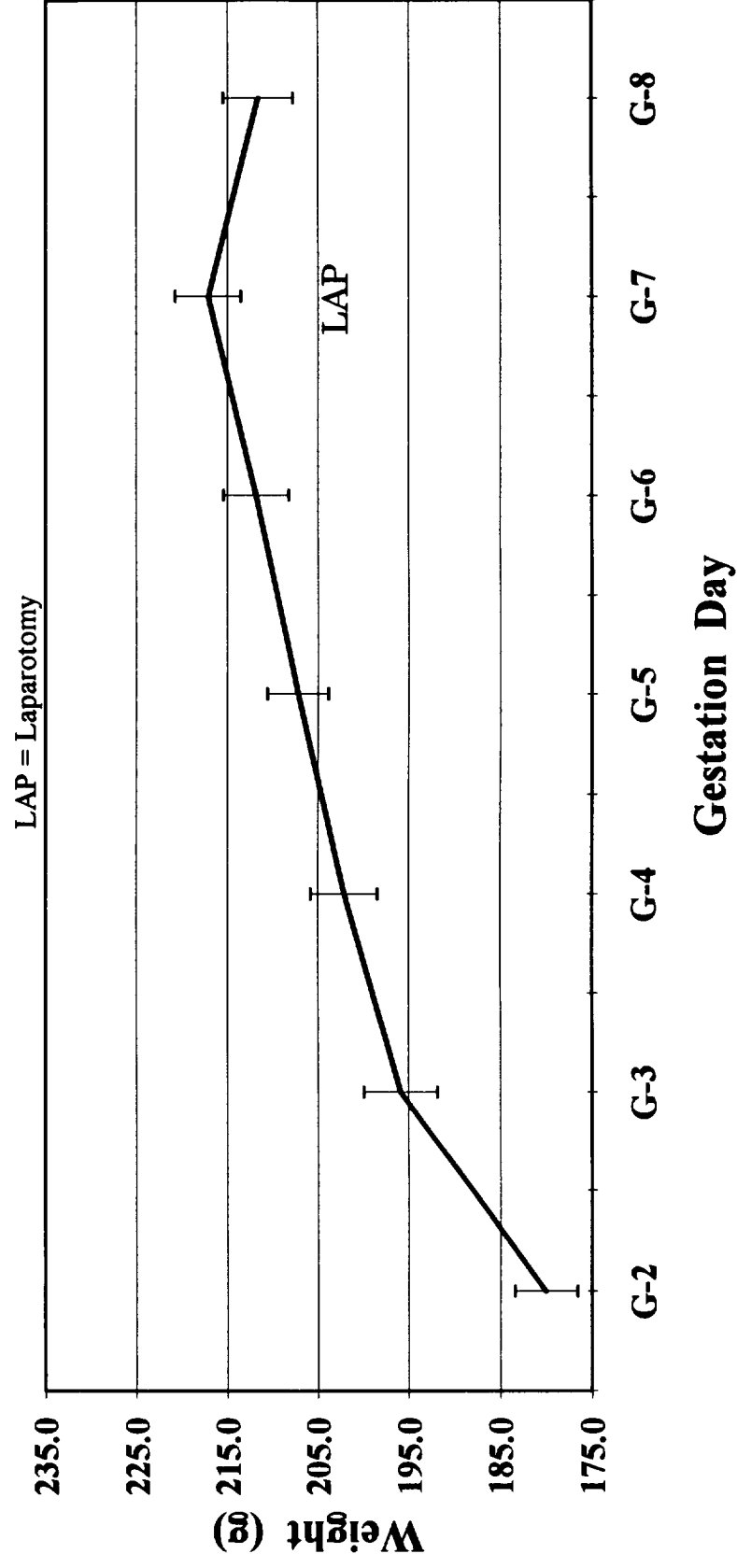


FIG.6 BODY WEIGHT (Mean +/- SEM) OF GROUP 4
(VIVARIUM CONTROL) ANIMALS n=10

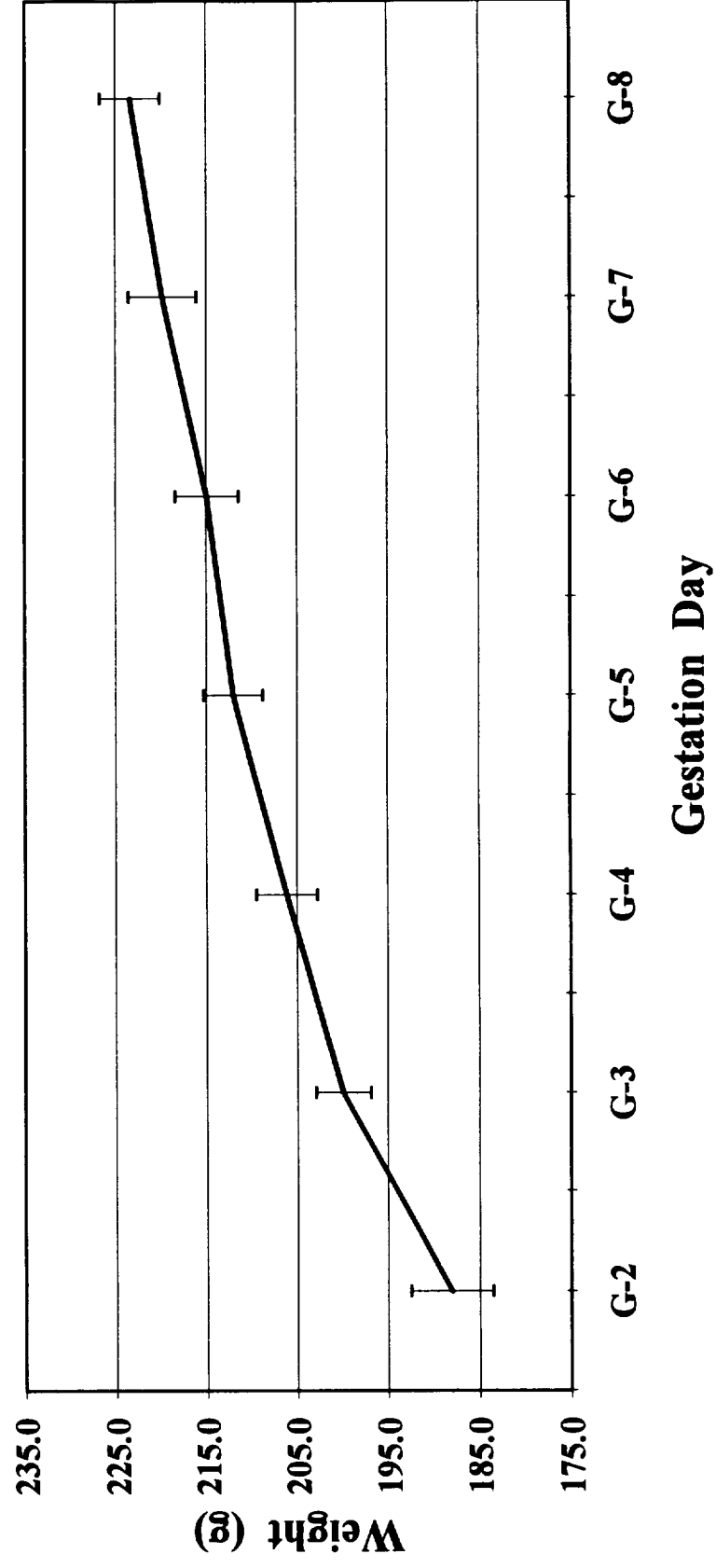
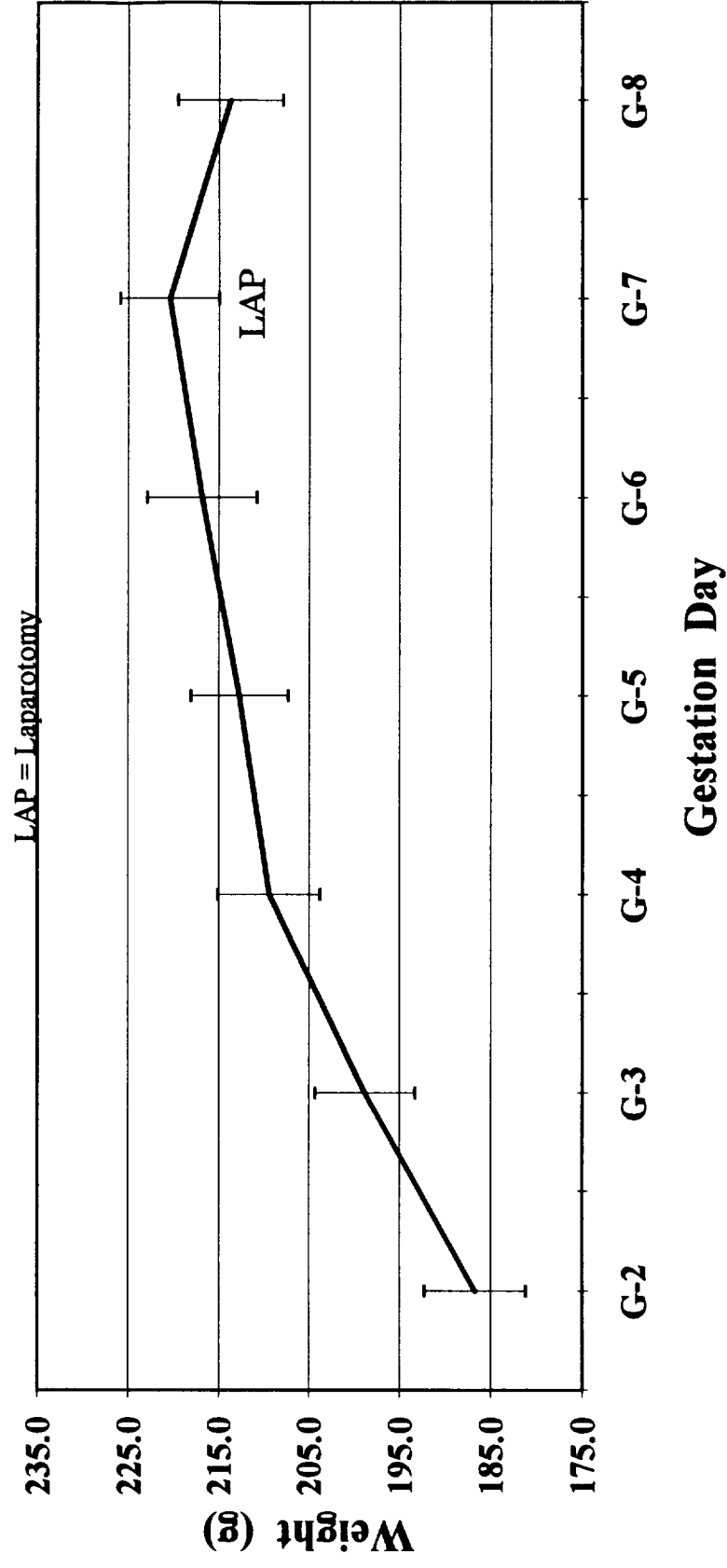
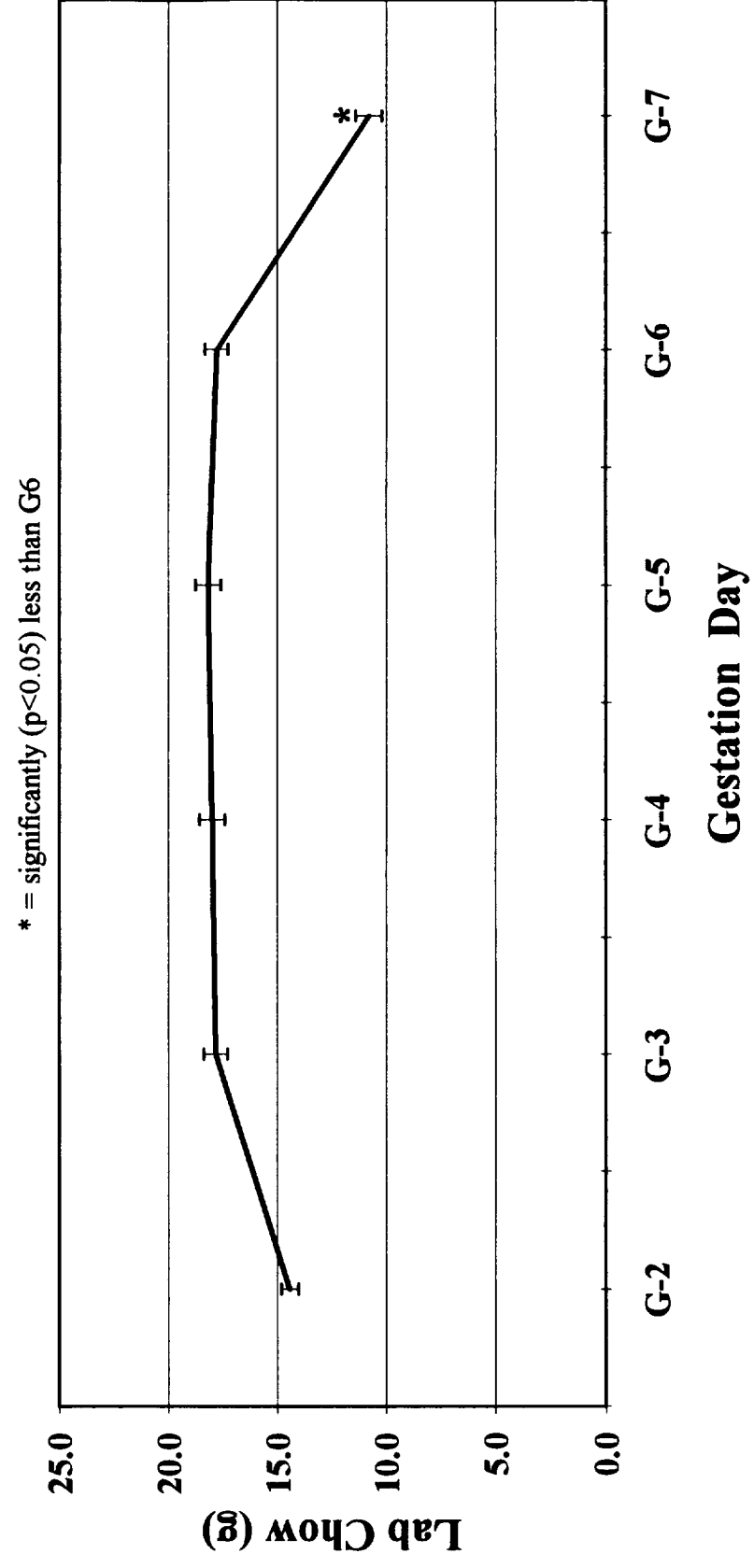


FIG.7 BODY WEIGHT (Mean \pm SEM) OF GROUP 5
(CESAREAN DELIVERY) ANIMALS n=10



**FIG.8 LAB CHOW CONSUMPTION (Mean +/- SEM) OF
ALL ANIMALS RECEIVED AT G2 n=80**



**FIG.9 LAB CHOW CONSUMPTION (Mean +/- SEM) OF
GROUP 1 ANIMALS n=10**

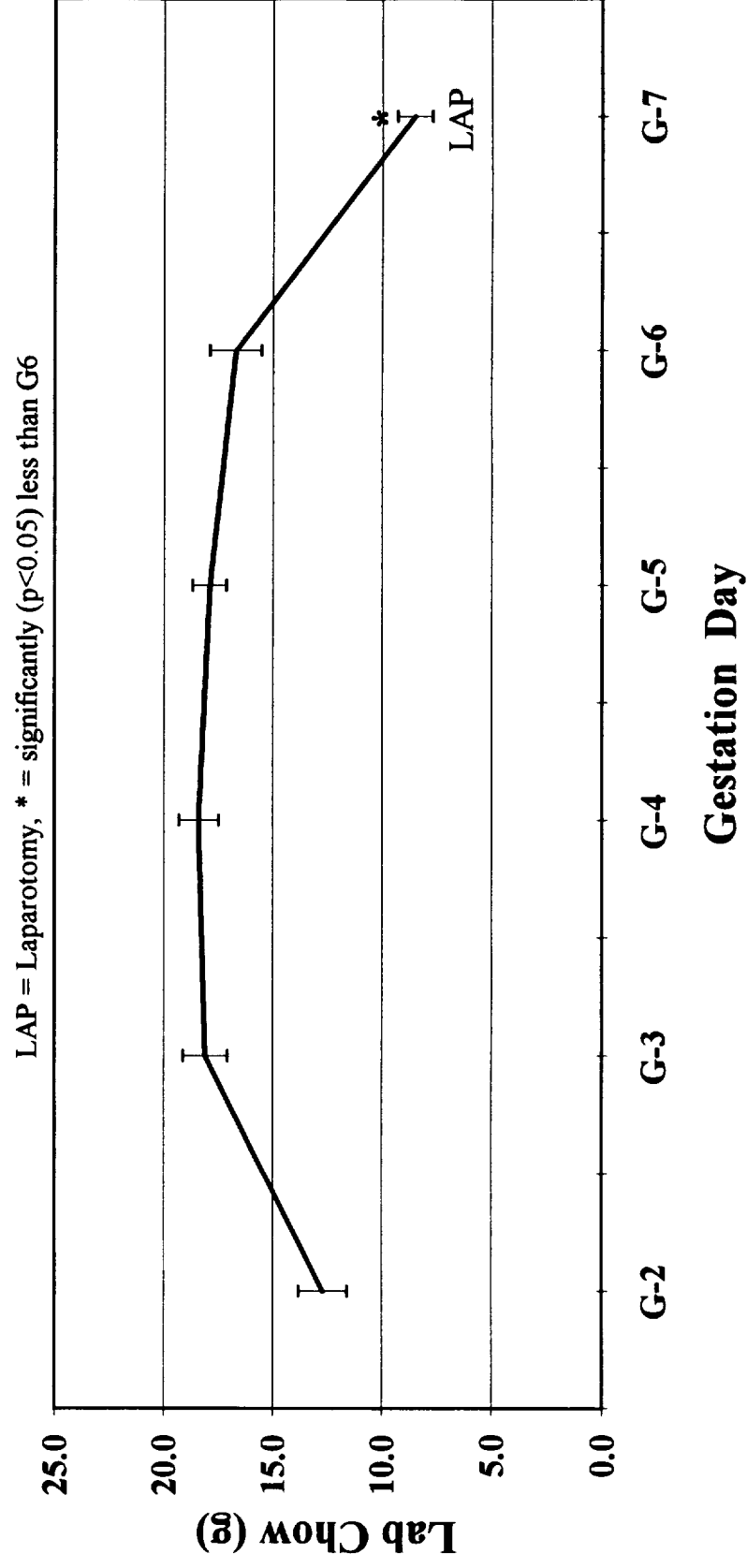
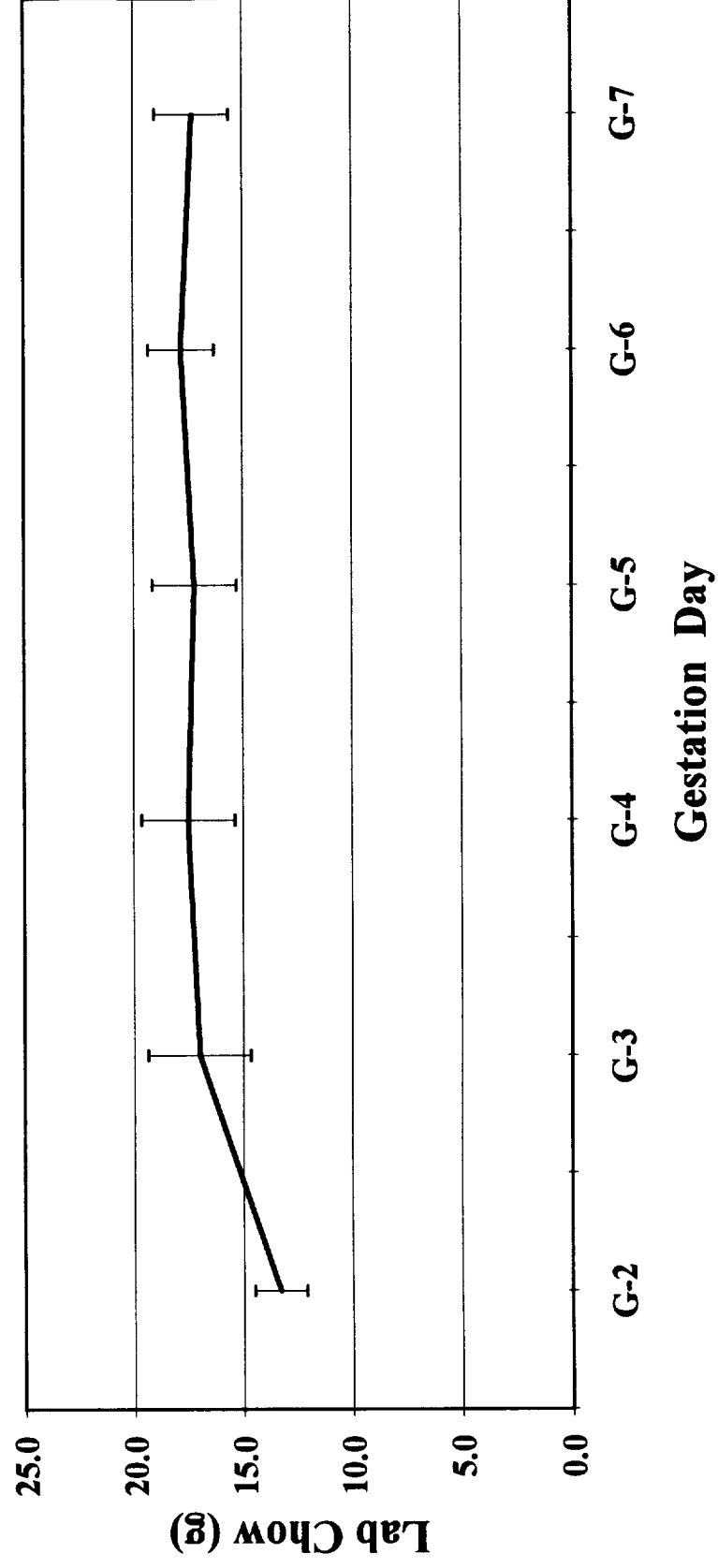


FIG.10 LAB CHOW CONSUMPTION (Mean +/- SEM) OF
GROUP 2 ANIMALS n=10



**FIG.11 LAB CHOW CONSUMPTION (Mean +/- SEM) OF
GROUP 3 ANIMALS n=10**

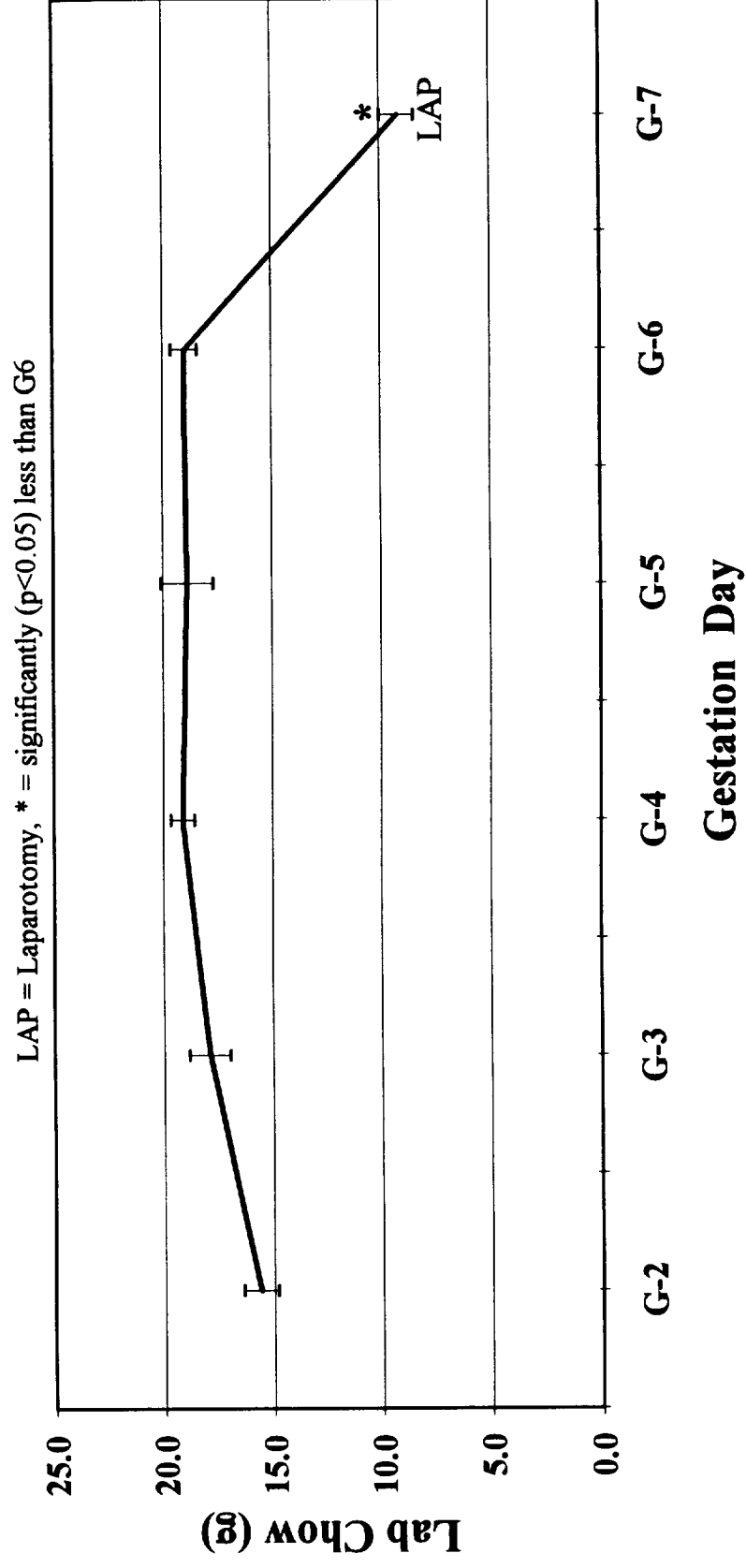


FIG.12 LAB CHOW CONSUMPTION (Mean +/- SEM) OF
GROUP 4 ANIMALS n=10

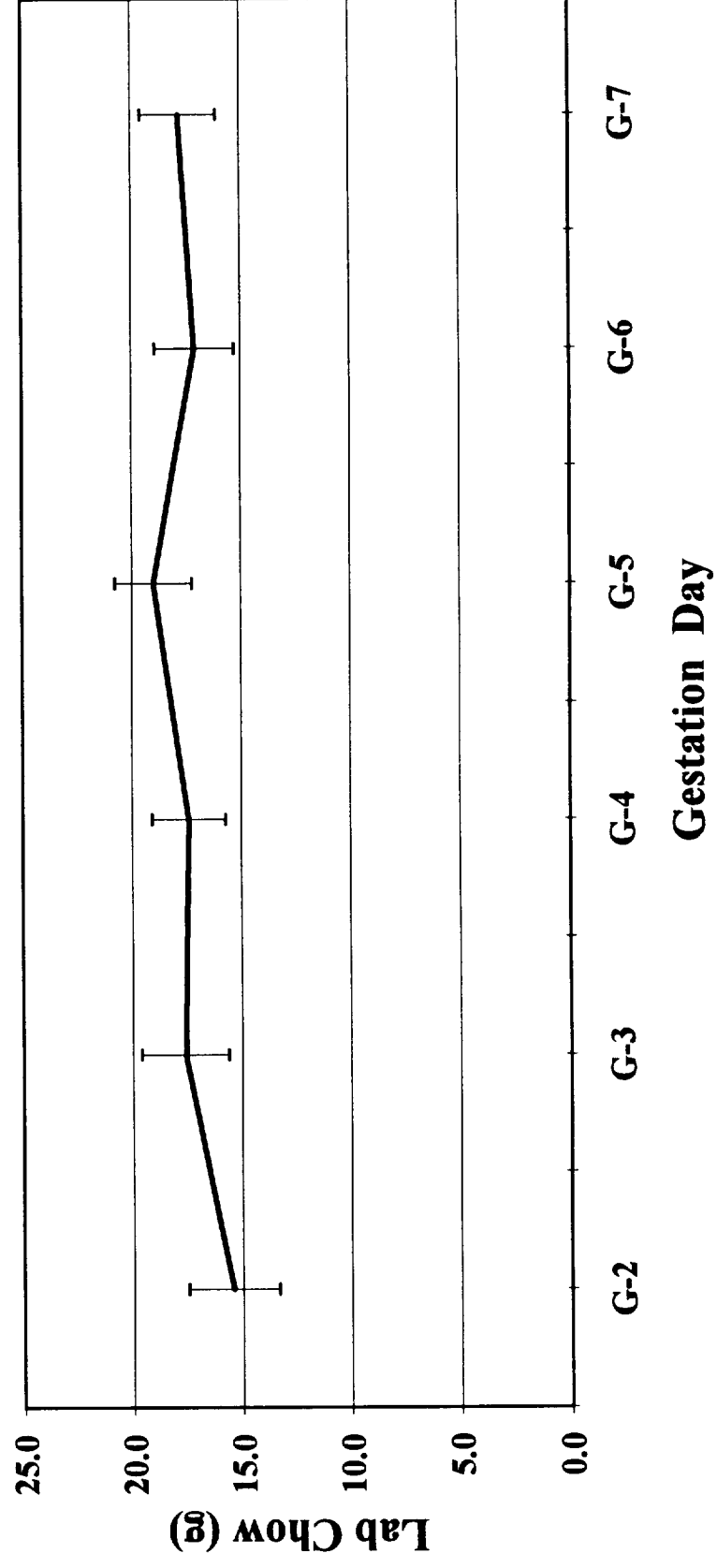
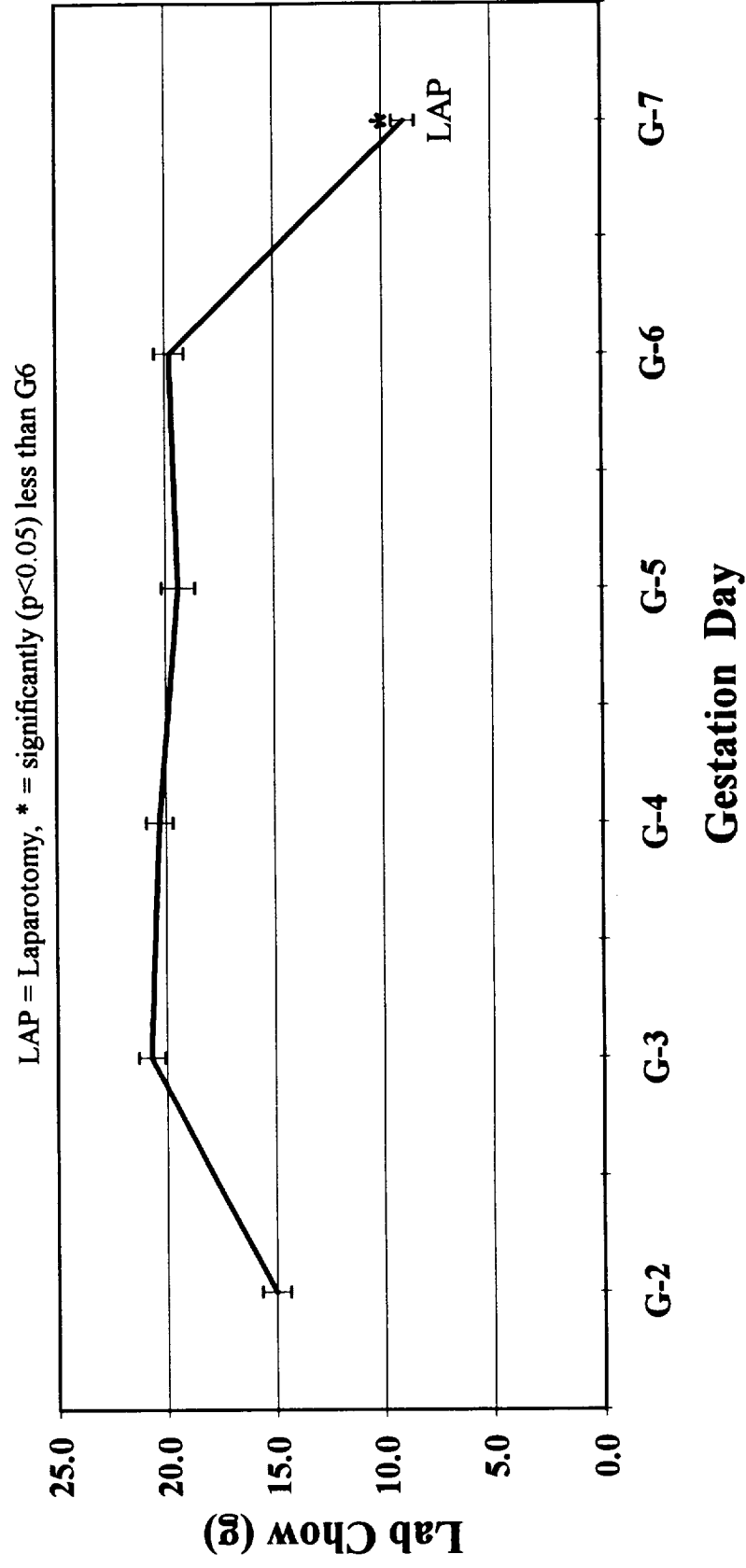
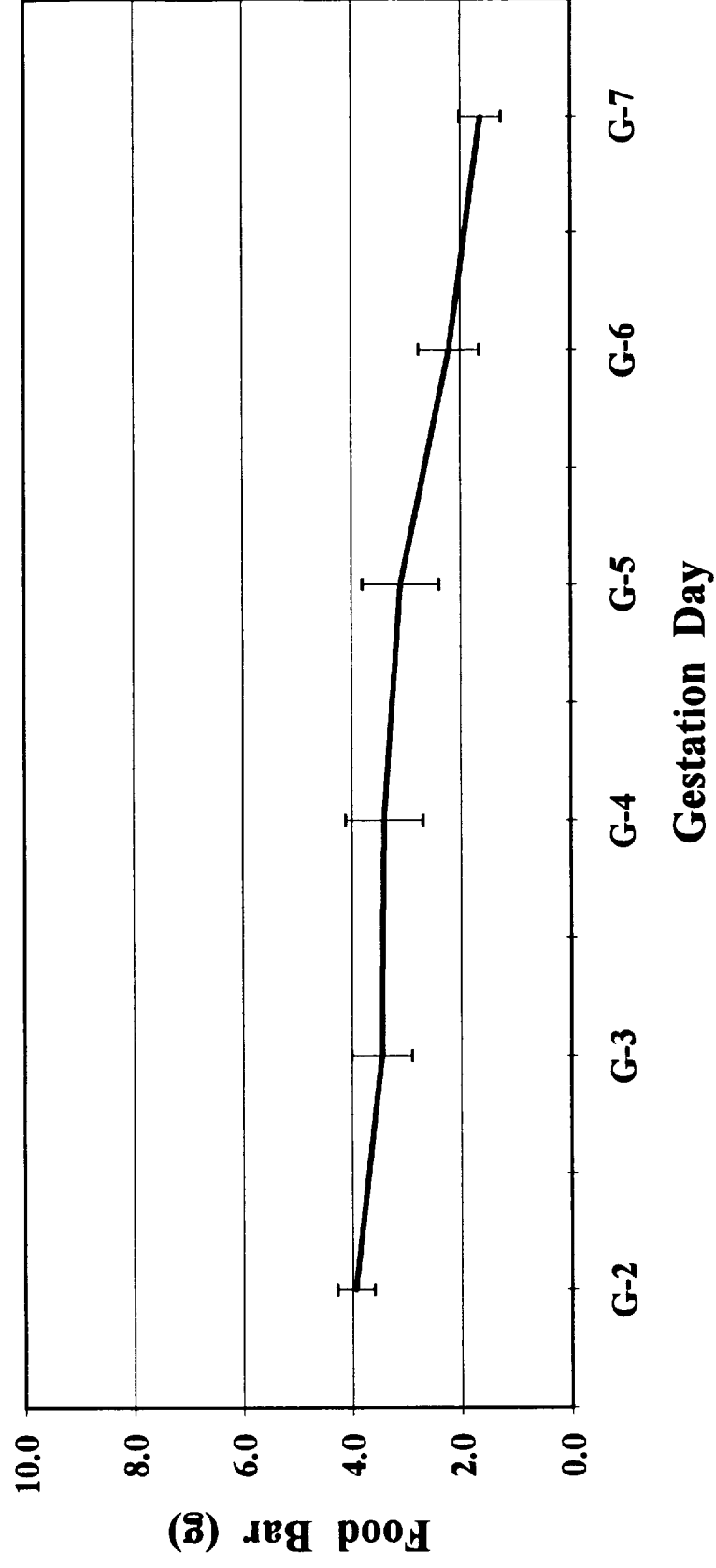


FIG.13 LAB CHOW CONSUMPTION (Mean +/- SEM) OF
GROUP 5 ANIMALS n=10



**FIG.14 FOOD BAR CONSUMPTION (Mean +/- SEM) OF
ALL ANIMALS RECEIVED AT G2 n=80**



**FIG.15 FOOD BAR CONSUMPTION (Mean +/- SEM) OF
GROUP 1 ANIMALS n=10**

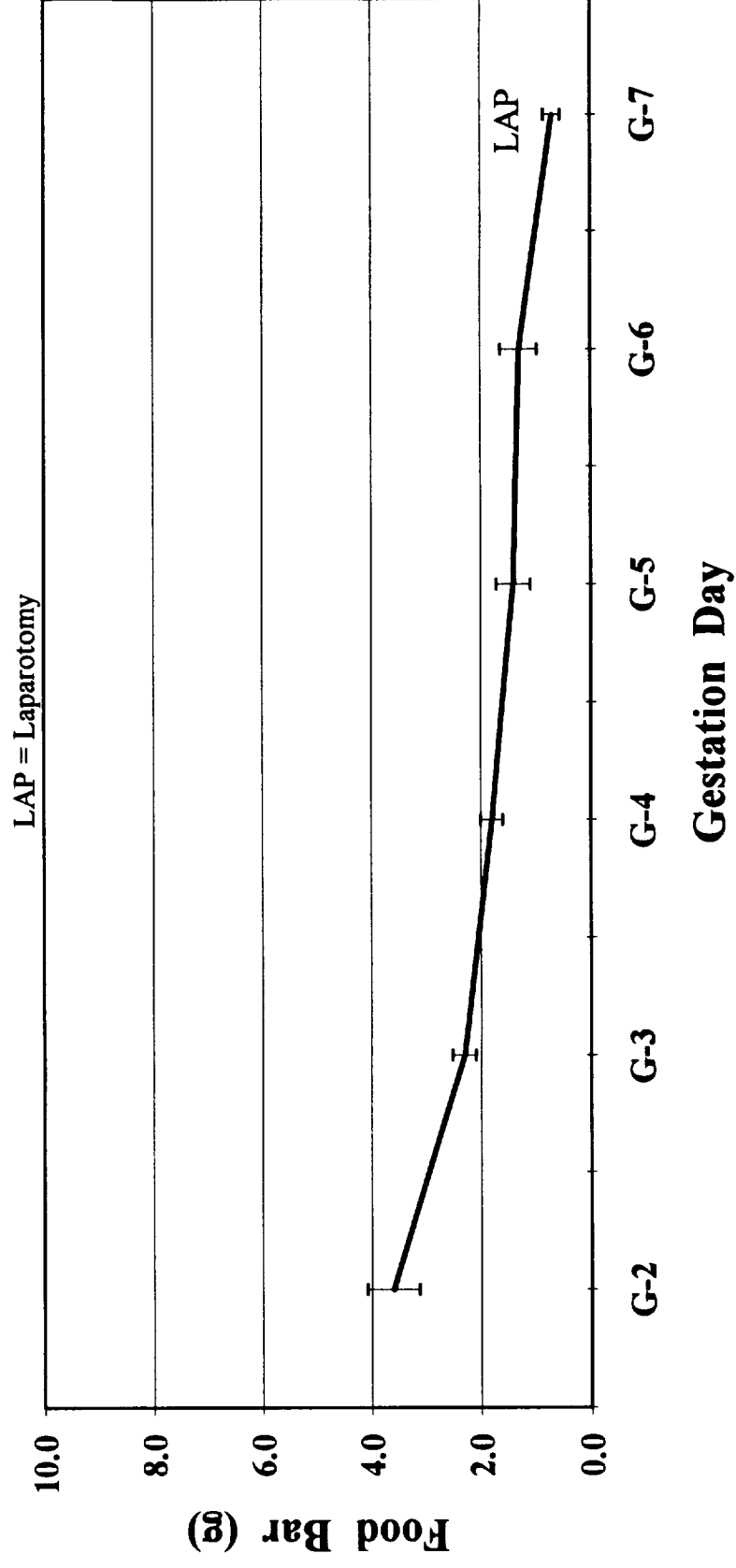


FIG.16 FOOD BAR CONSUMPTION (Mean \pm SEM) OF
GROUP 2 ANIMALS n=10

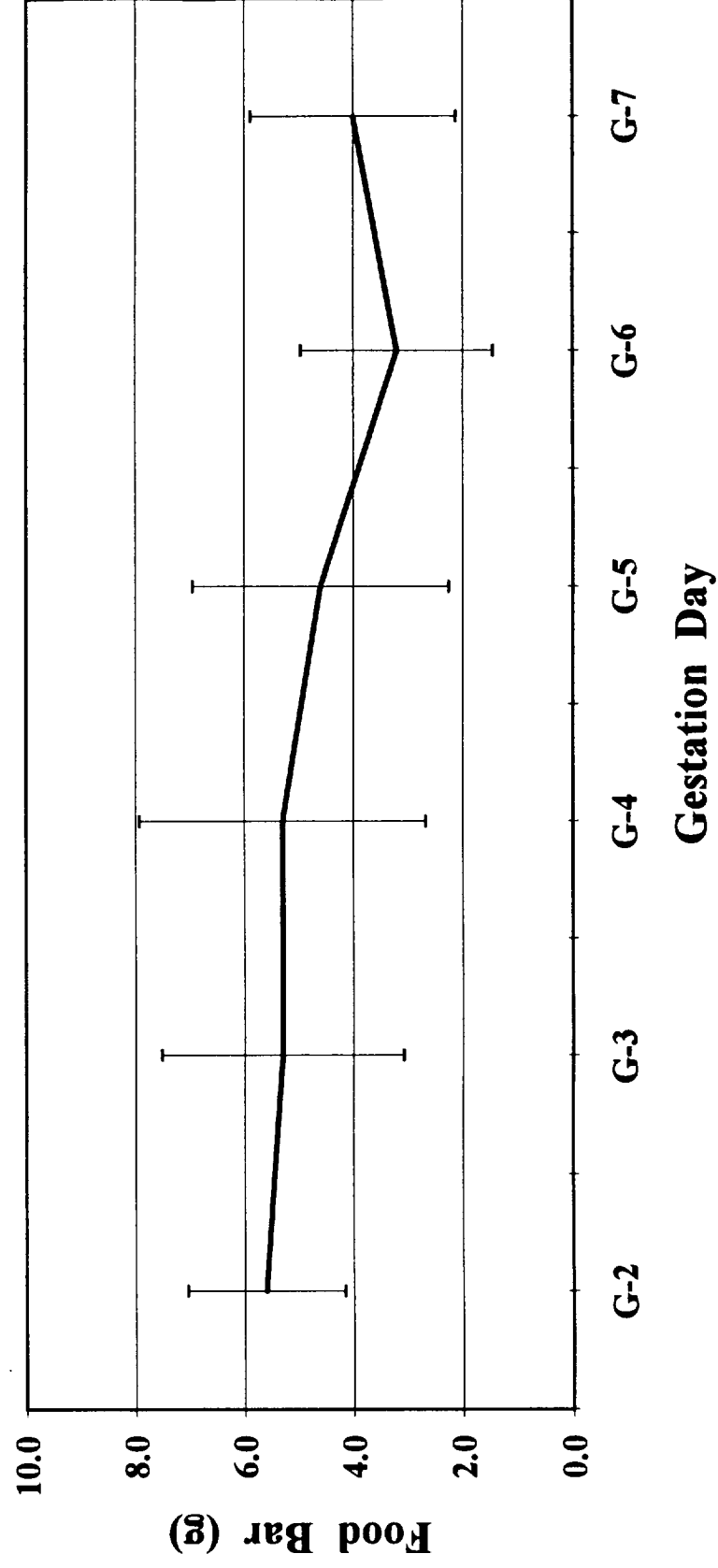
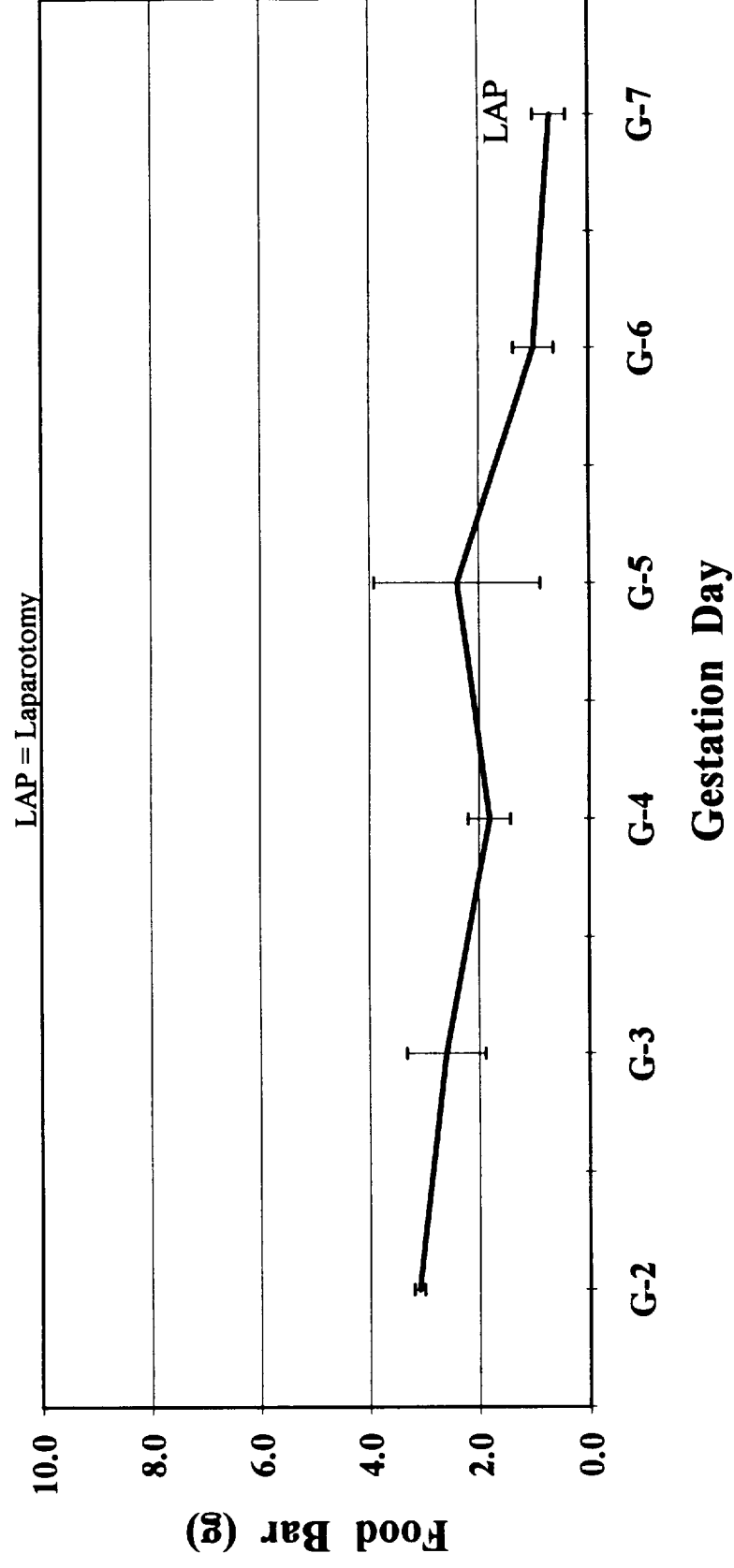
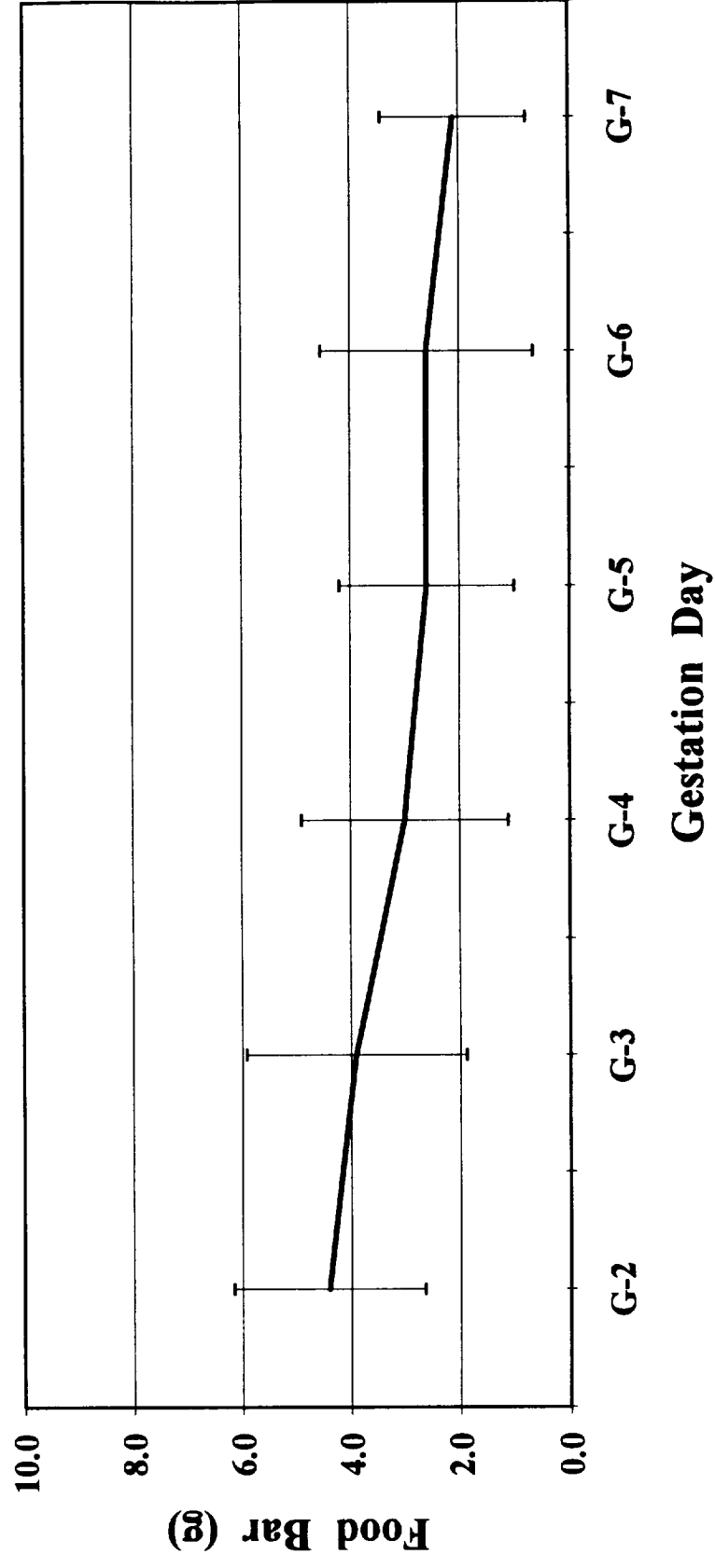


FIG.17 FOOD BAR CONSUMPTION (Mean +/- SEM) OF
GROUP 3 ANIMALS n=10



**FIG.18 FOOD BAR CONSUMPTION (Mean +/- SEM) OF
GROUP 4 ANIMALS n=10**



**FIG.19 FOOD BAR CONSUMPTION (Mean +/- SEM) OF
GROUP 5 ANIMALS n=10**

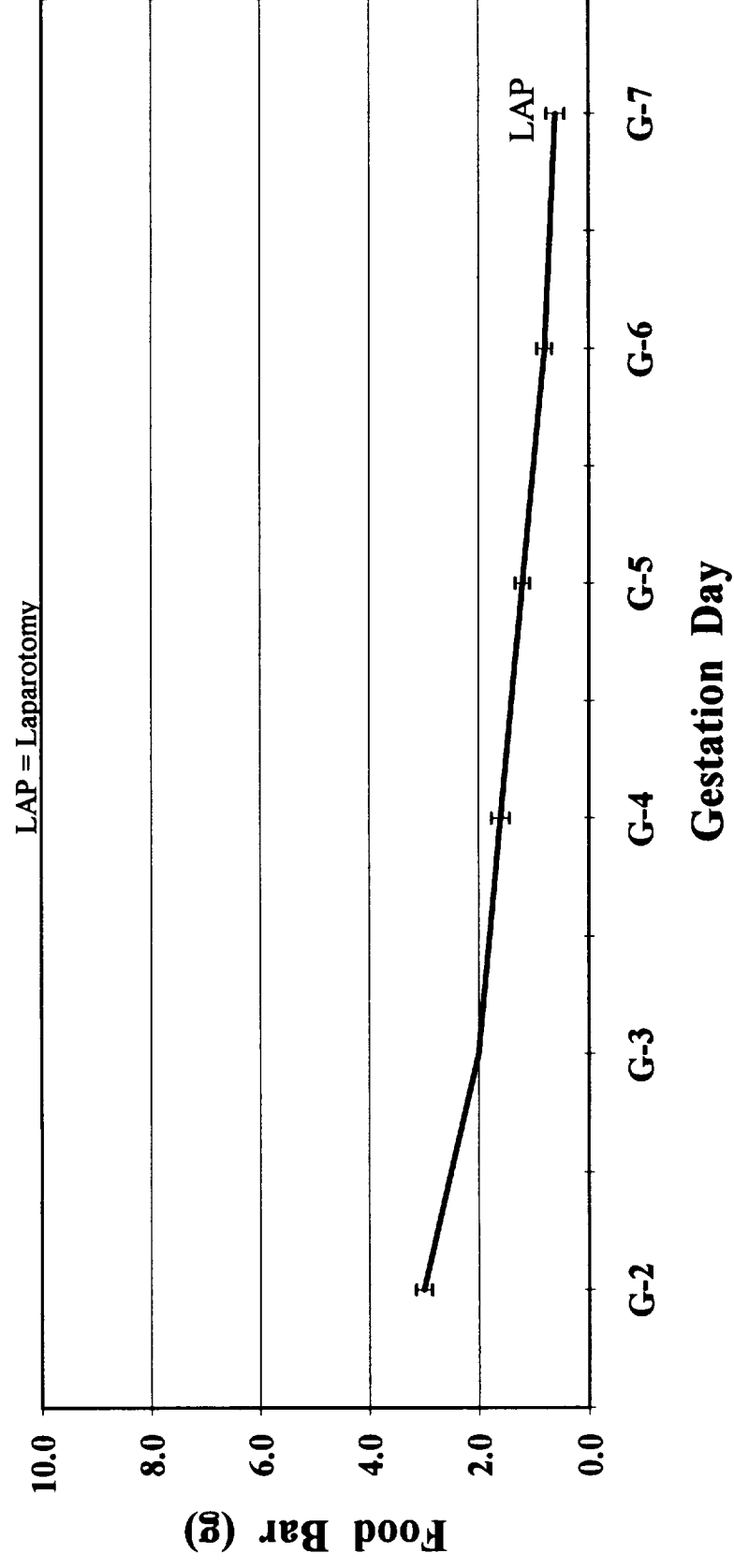
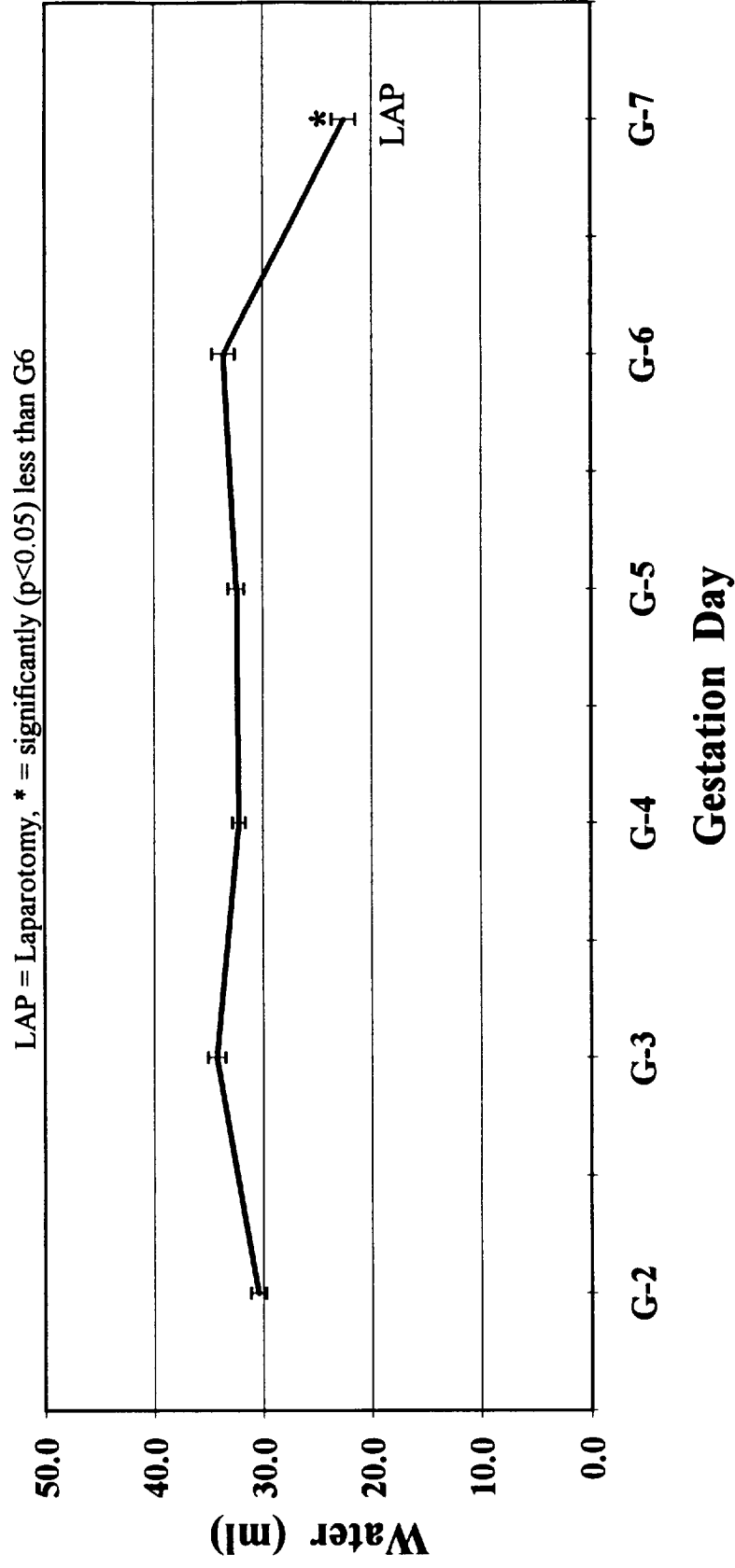
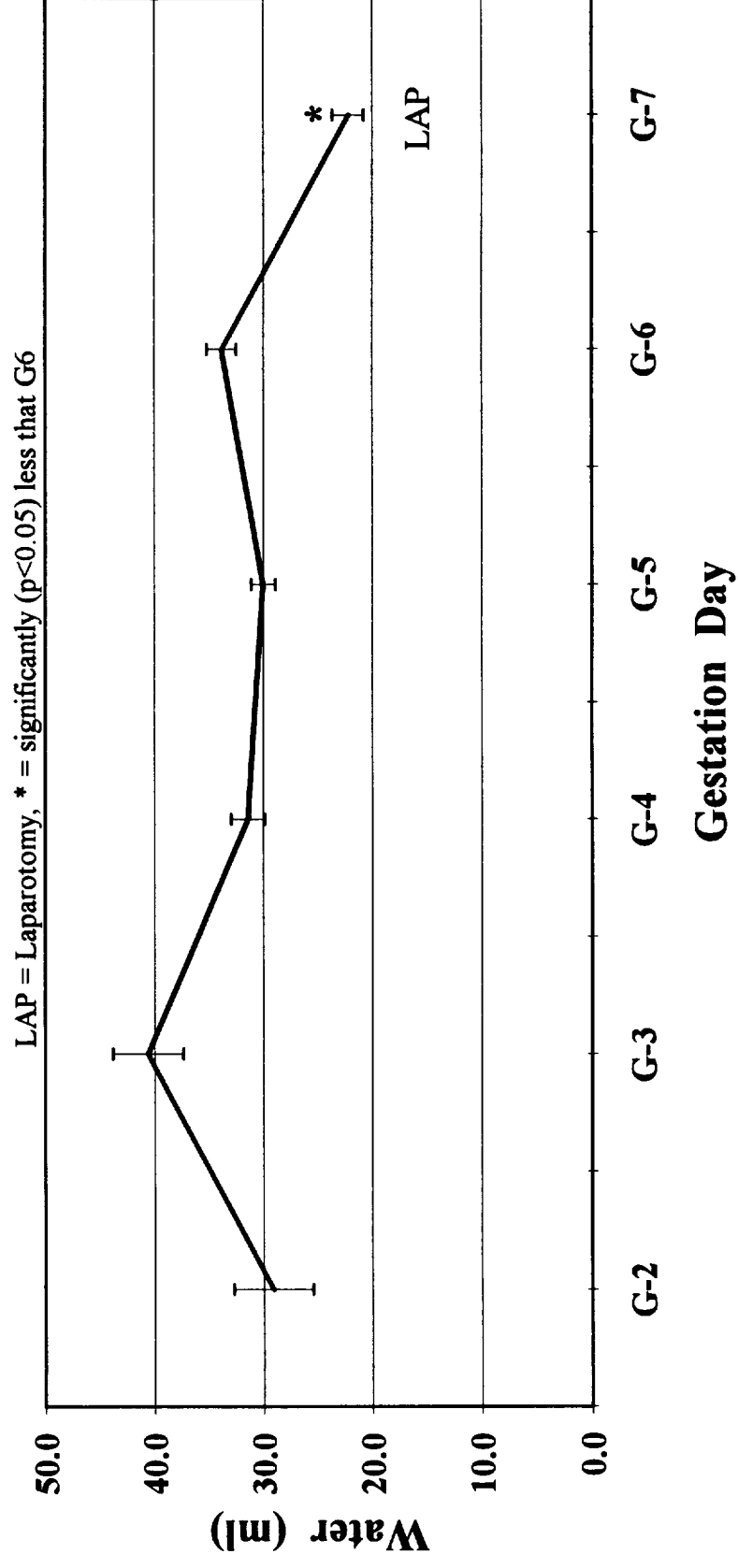


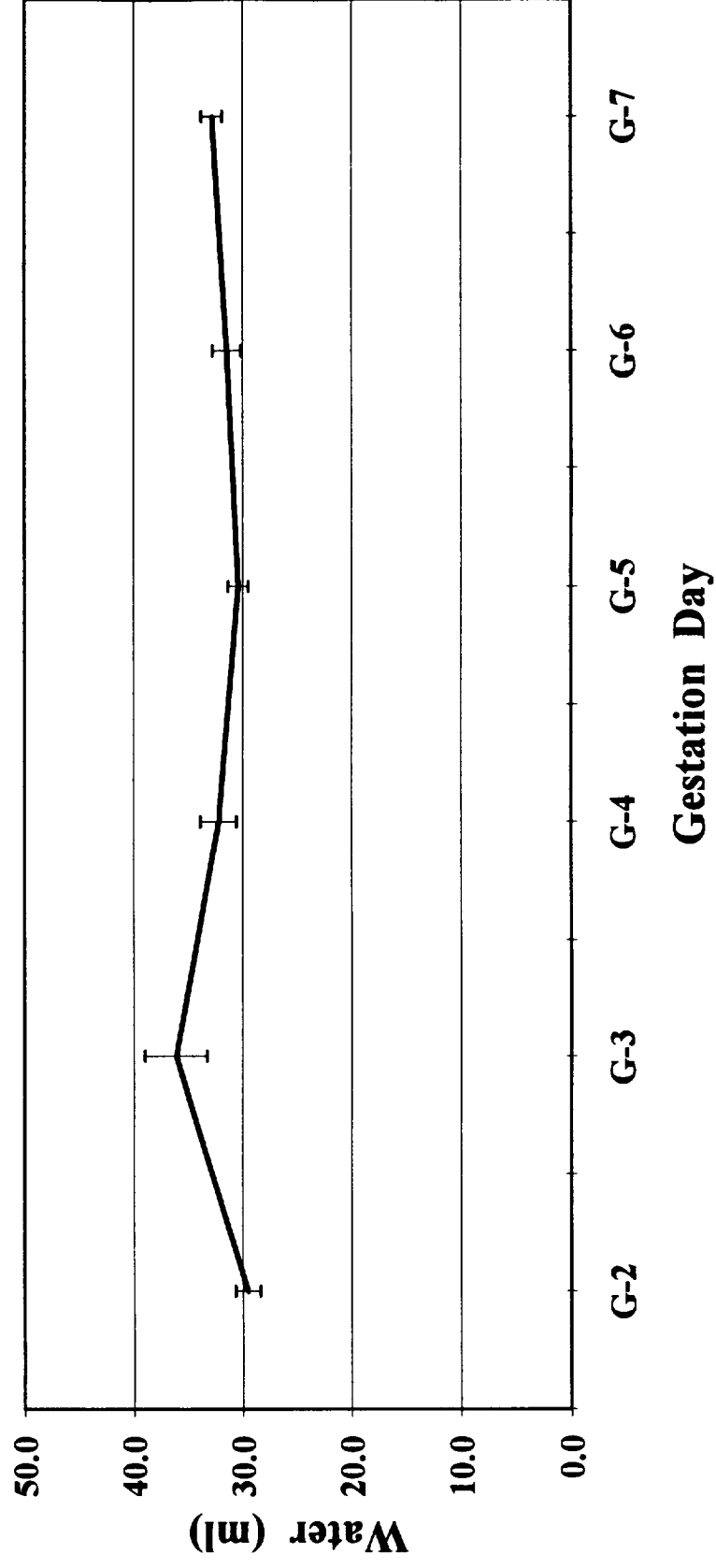
FIG.20 WATER CONSUMPTION (Mean +/- SEM) OF ALL ANIMALS RECEIVED AT G2 n=80



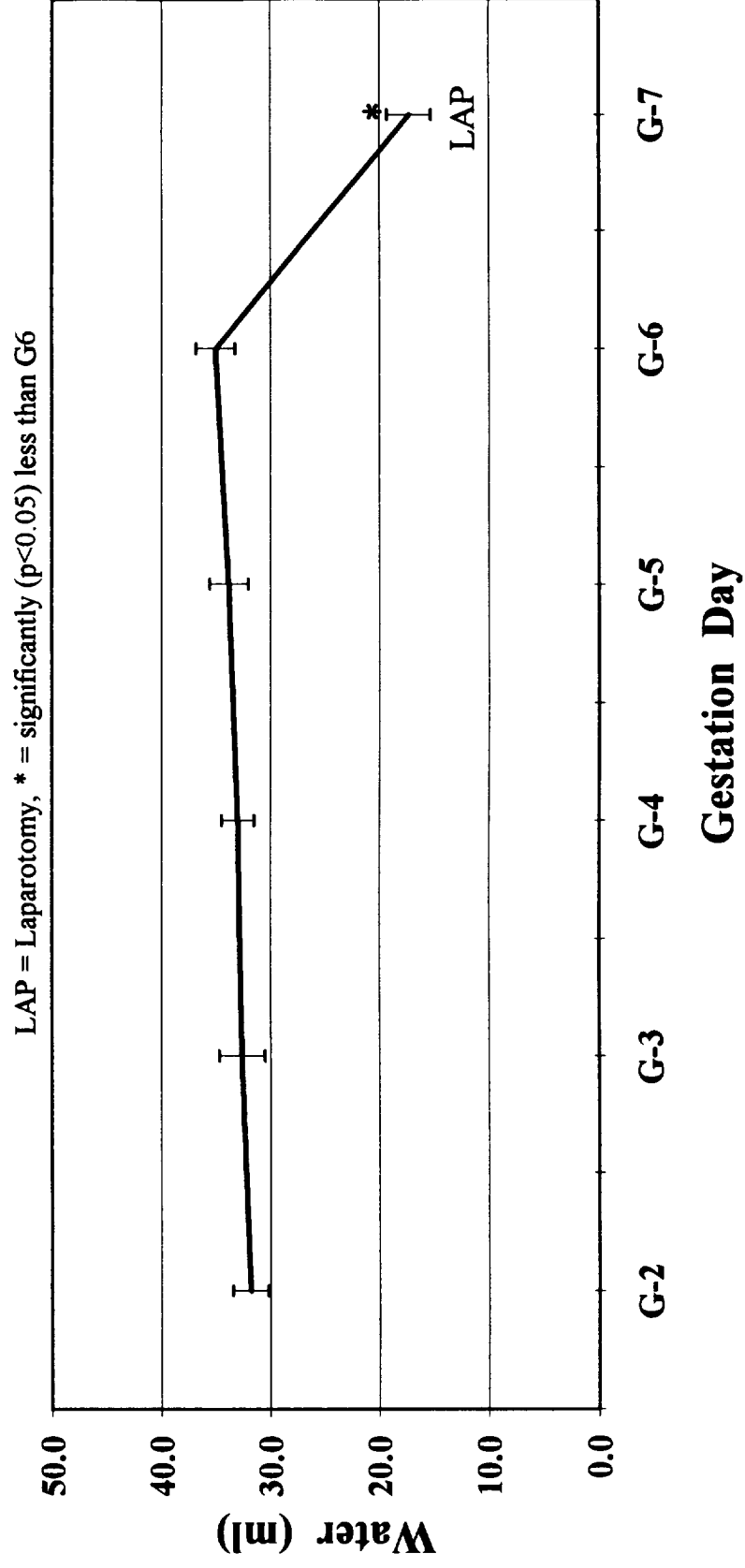
**FIG.21 WATER CONSUMPTION (Mean +/- SEM) OF
GROUP 1 ANIMALS n=10**



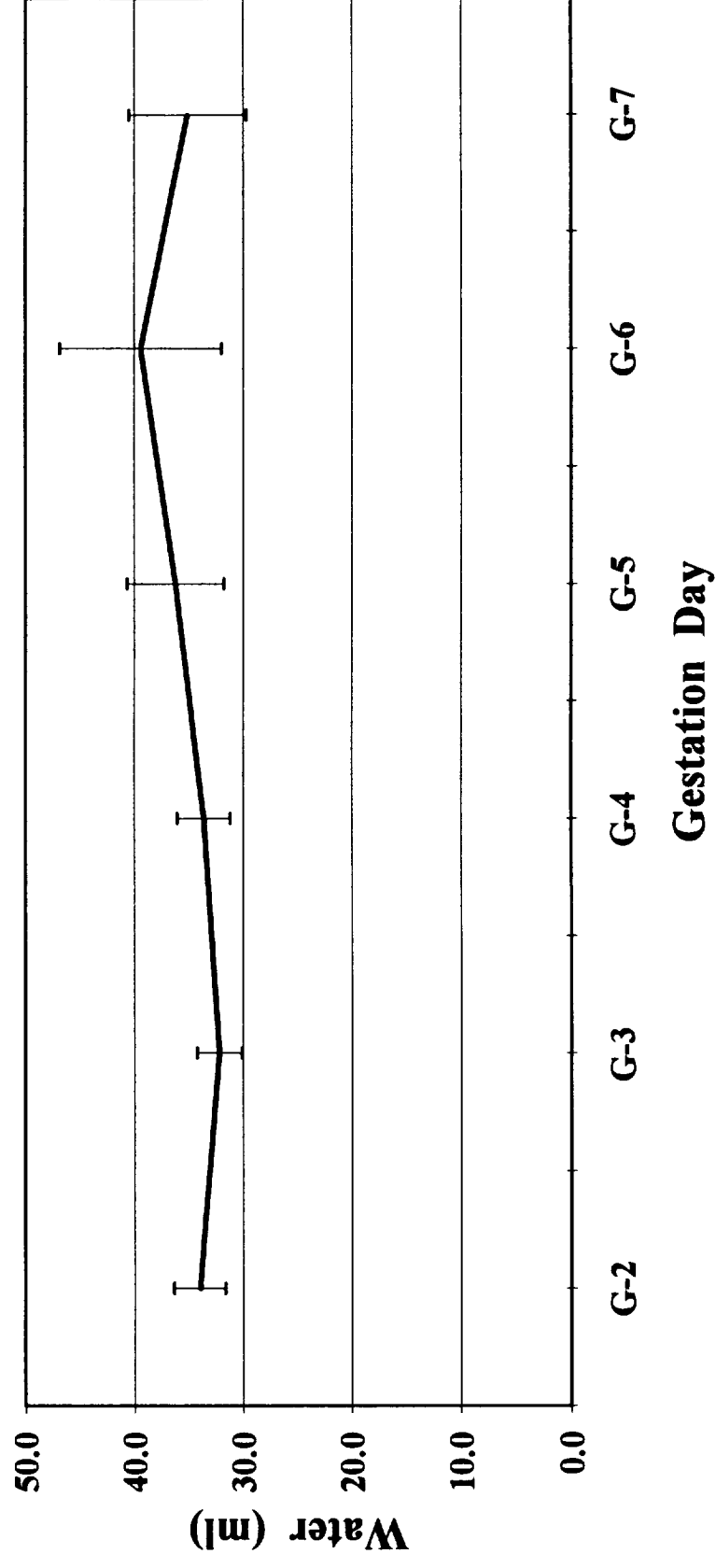
**FIG.22 WATER CONSUMPTION (Mean +/- SEM) OF
GROUP 2 ANIMALS n=10**



**FIG.23 WATER CONSUMPTION (Mean +/- SEM) OF
GROUP 3 ANIMALS n=10**



**FIG.24 WATER CONSUMPTION (Mean \pm SEM) OF
GROUP 4 ANIMALS n=10**



**FIG.25 WATER CONSUMPTION (Mean +/- SEM) OF
GROUP 5 ANIMALS n=10**

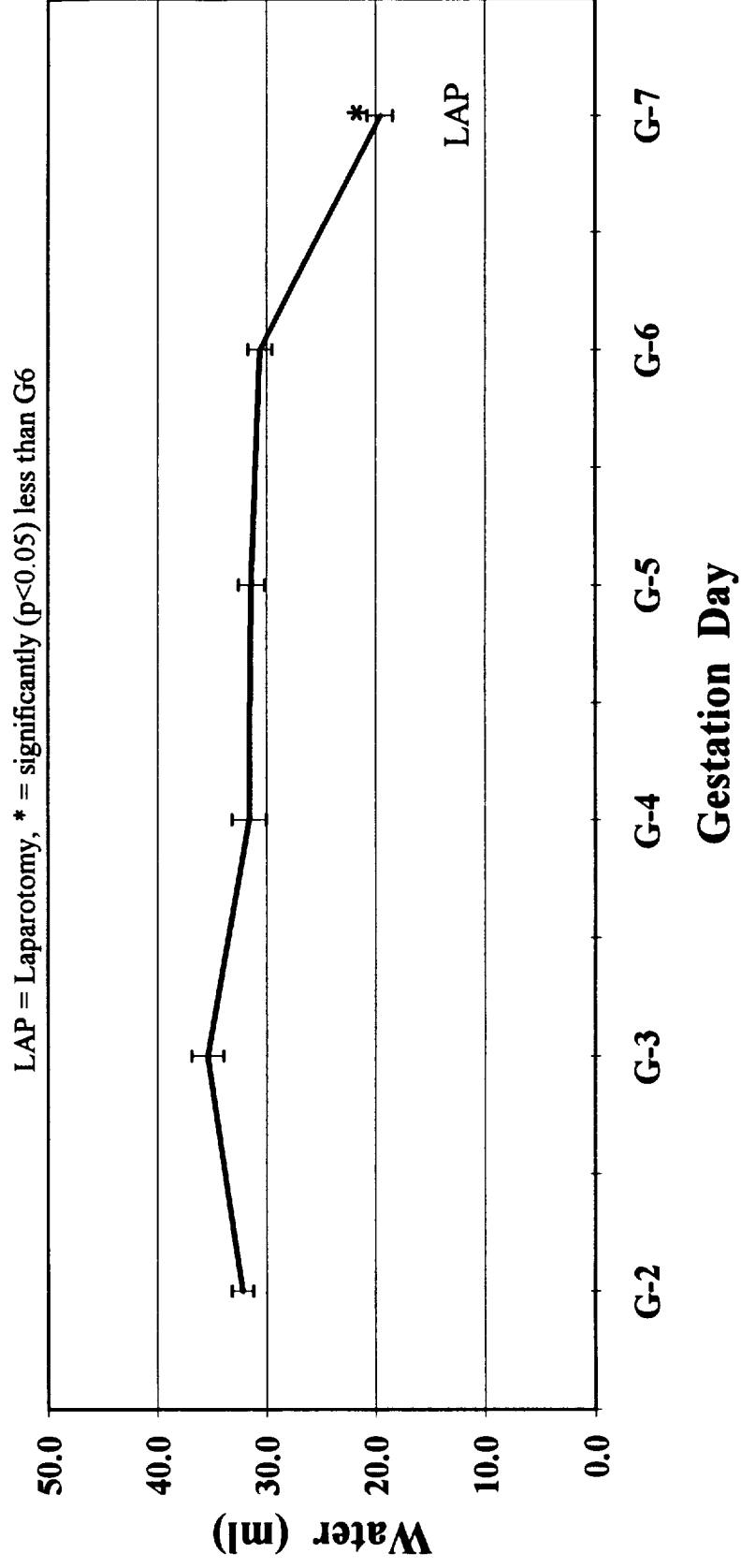


FIG.26 DECIDUAL SWELLINGS IN THE THREE TEST GROUPS LAPAROTOMIZED AT G7 n=10/group

(Mean +/- SEM)

* = significantly ($p < 0.05$) more than the left horn, Nominal Flight

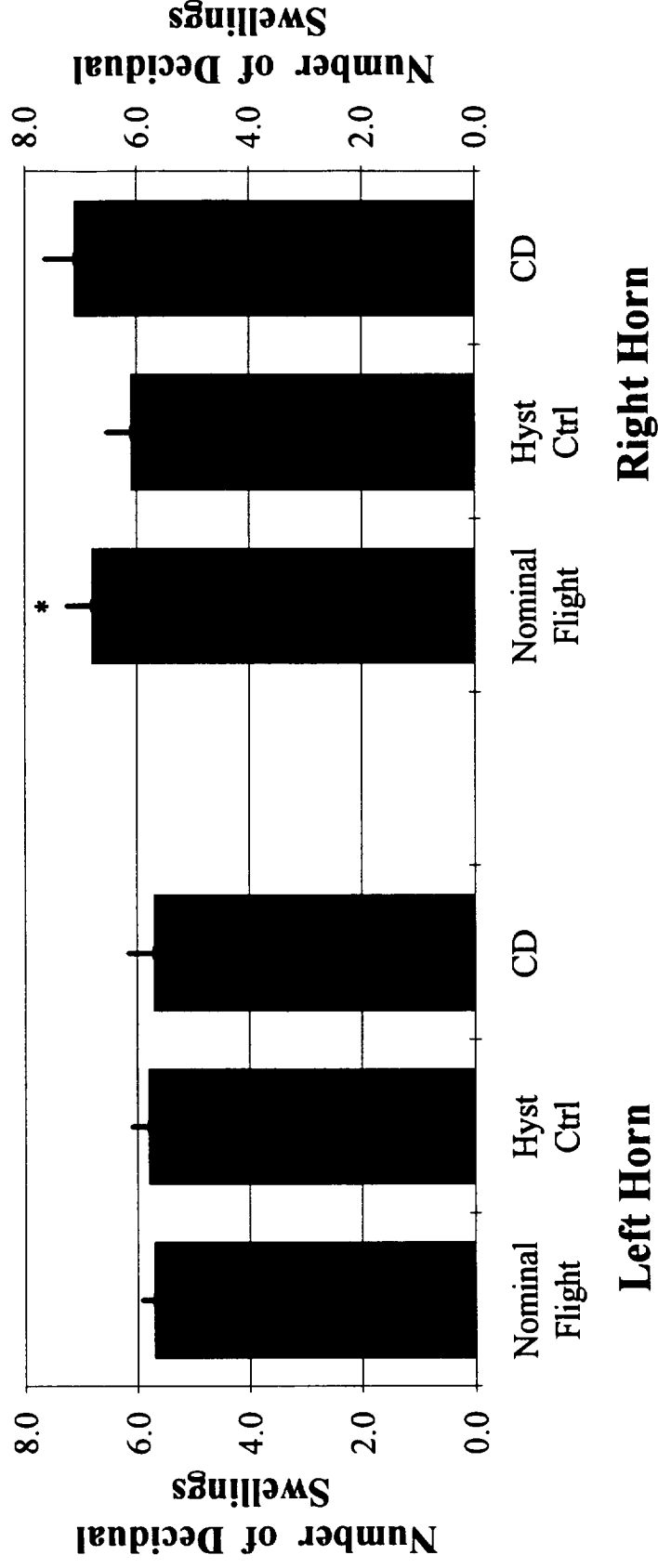
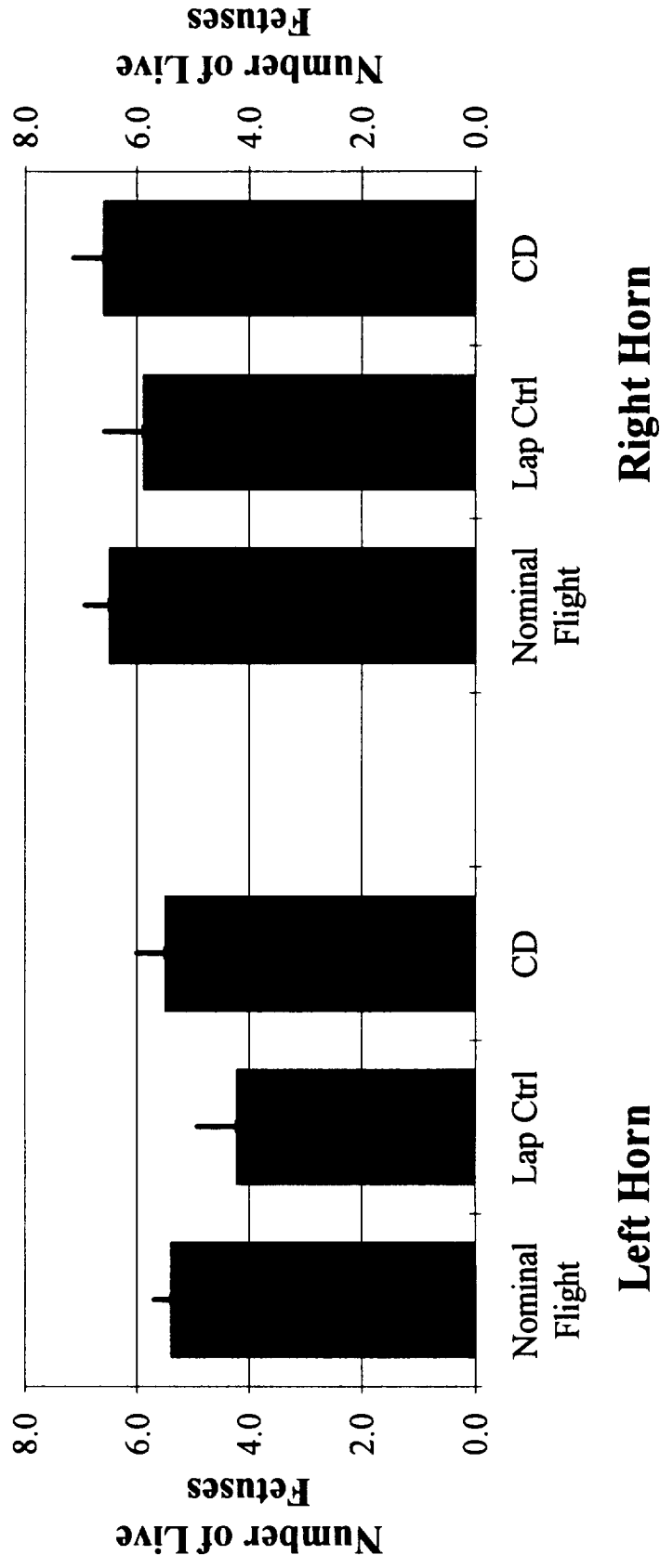


FIG.27 LIVE FETUSES IN THE THREE TEST GROUPS
UNILATERALLY HYSTERECTOMIZED AT G17
n=10/group (Mean +/- SEM)



**FIG.28 DECIDUAL SWELLINGS AT G7 COMPARED TO
LIVE FETUSES AT G17 IN THE SAME ANIMAL**
n=10/group (Mean +/- SEM)

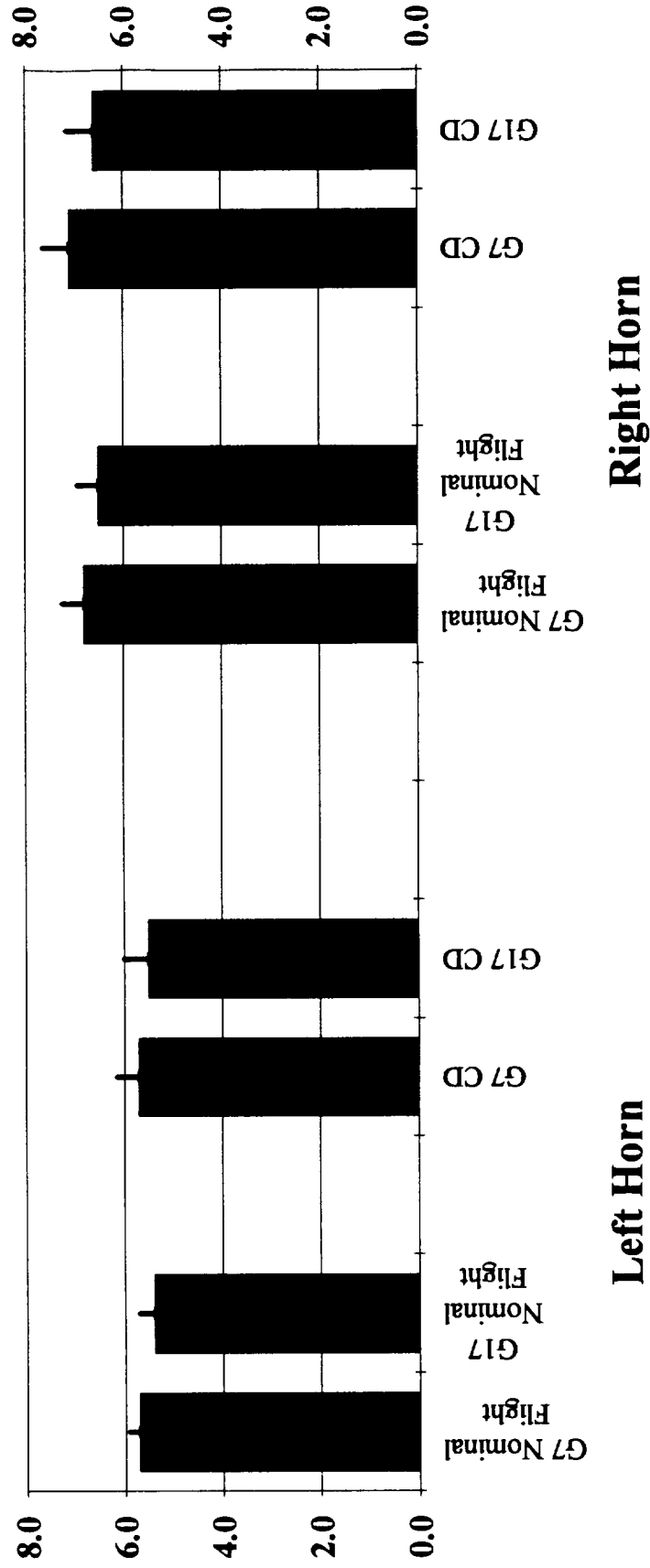


FIG.29 CROWN - RUMP LENGTH (Mean +/- SEM) AT G17
UNILATERAL HYSTERECTOMY

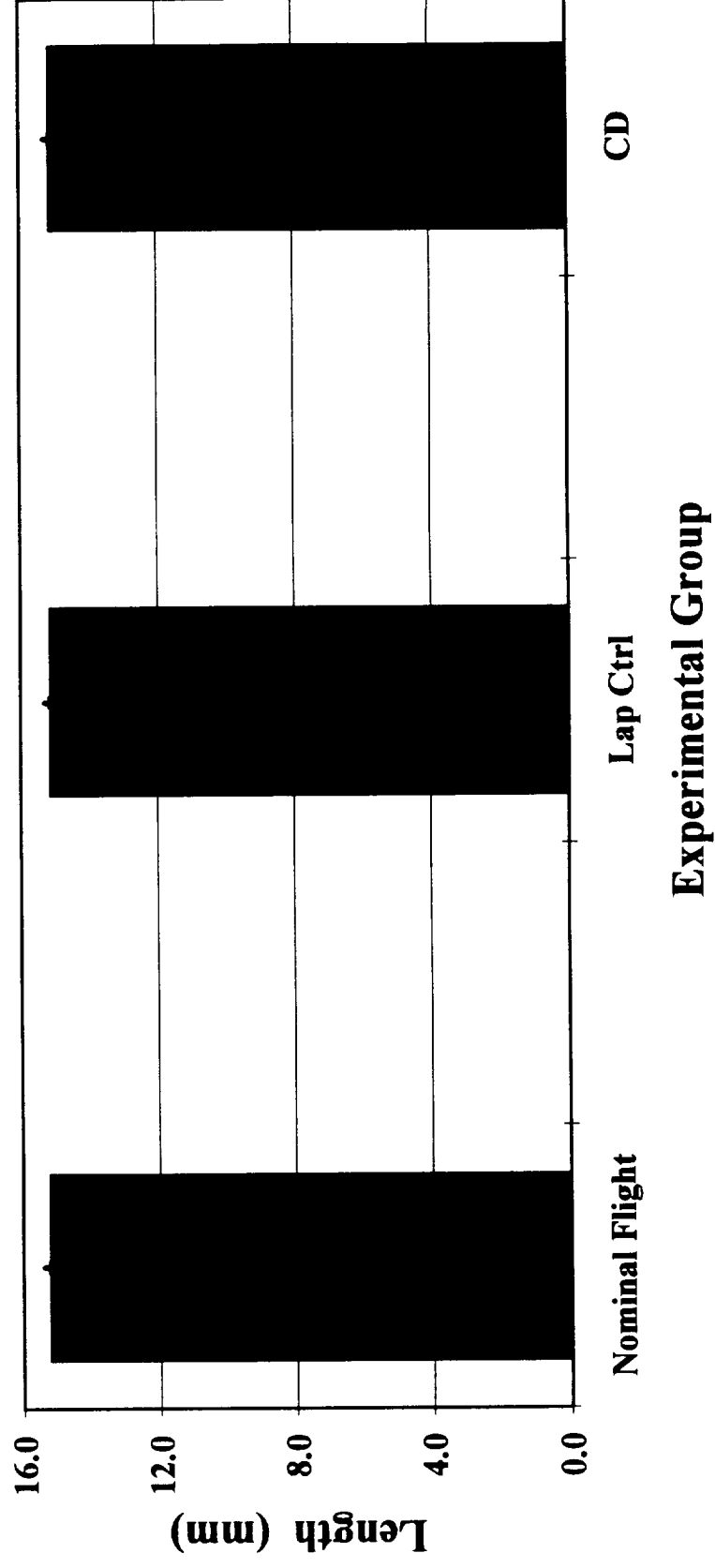


FIG.30 WEIGHT OF FETUSES (Mean +/- SEM) AT G17
UNILATERAL HYSTERECTOMY

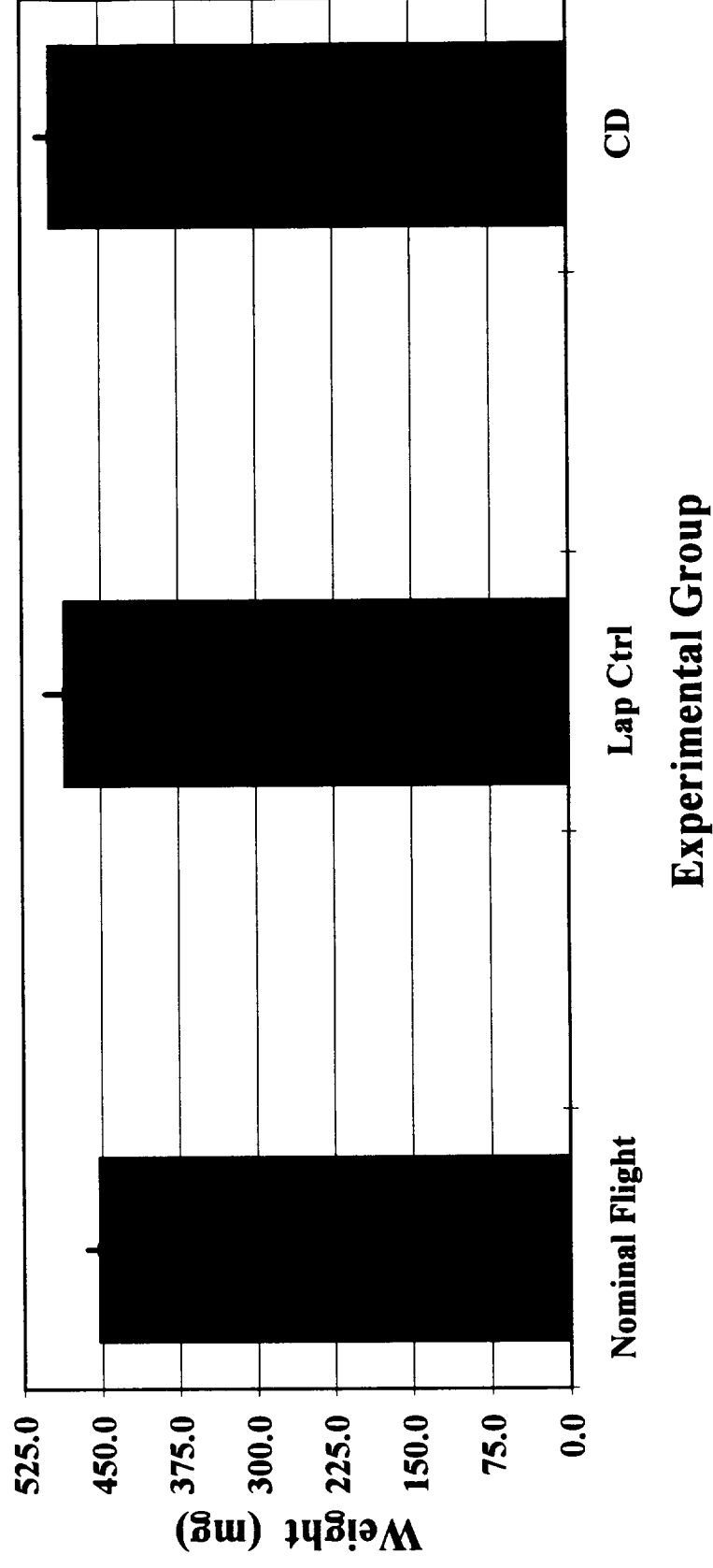
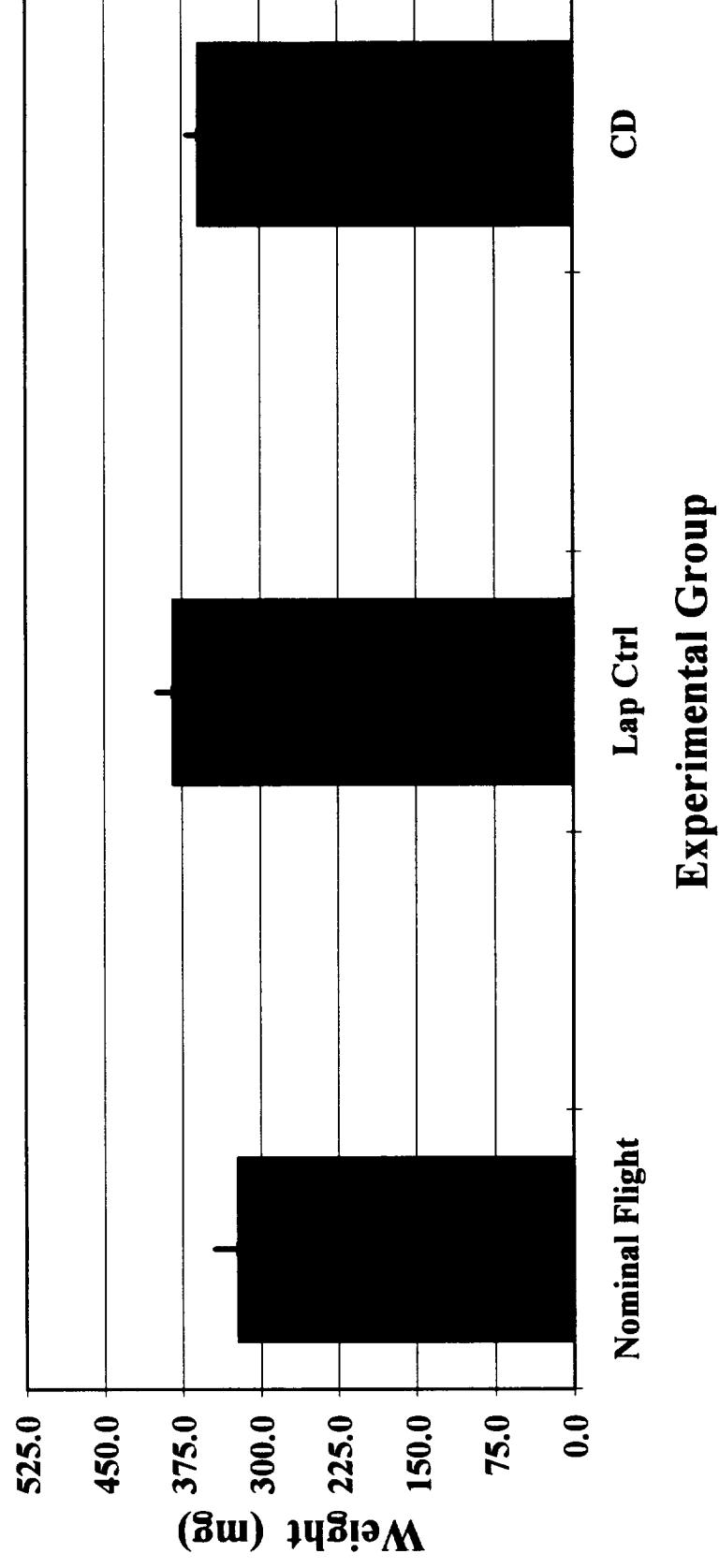
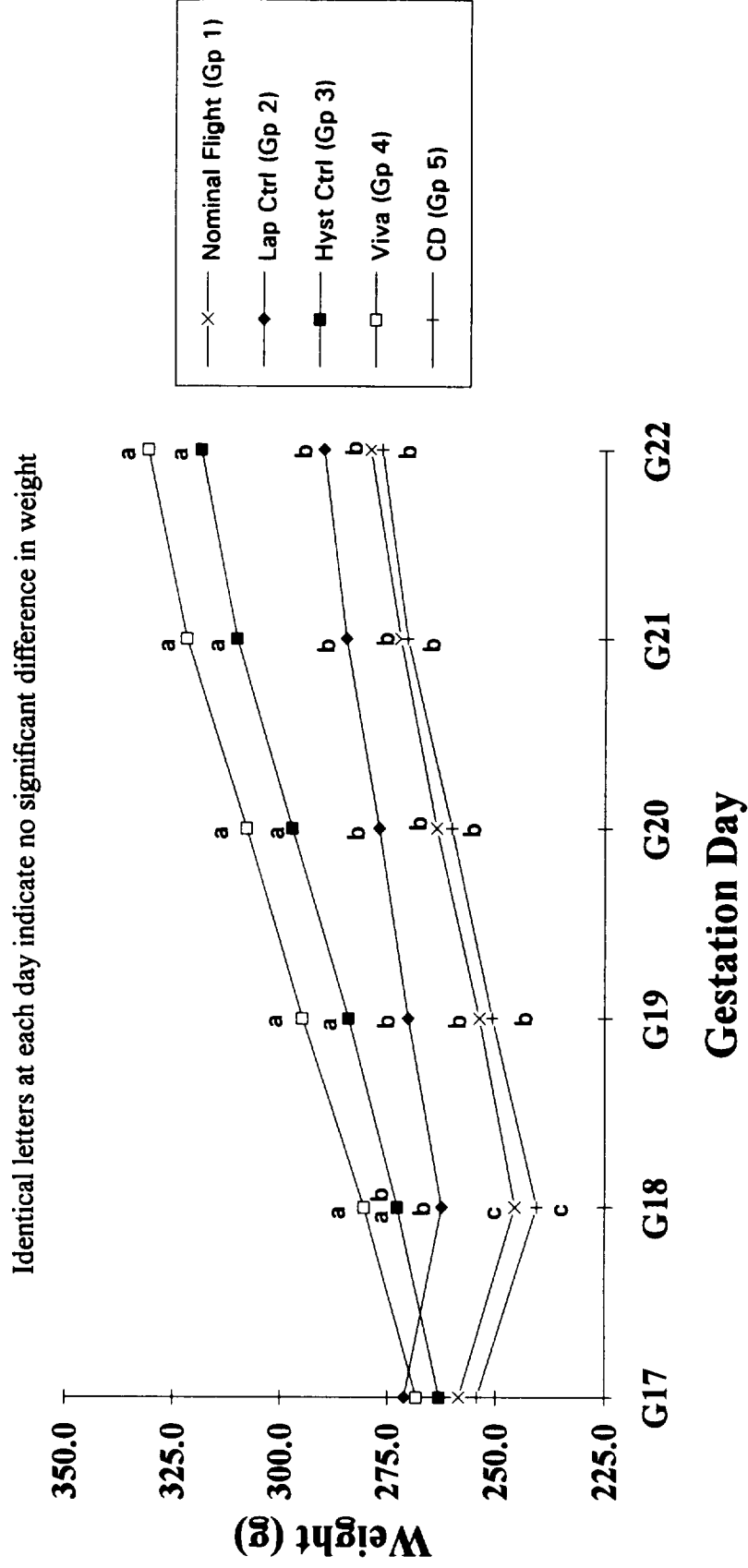


FIG.31 WEIGHT OF PLACENTAS (Mean +/- SEM) AT G17
UNILATERAL HYSTERECTOMY



**FIG.32 BODY WEIGHT (Mean +/- SEM) OF PREGNANT
RATS G17 - G22**



**FIG.33 FOOD BAR CONSUMED (Mean \pm SEM) BY
PREGNANT RATS G17 - G21**

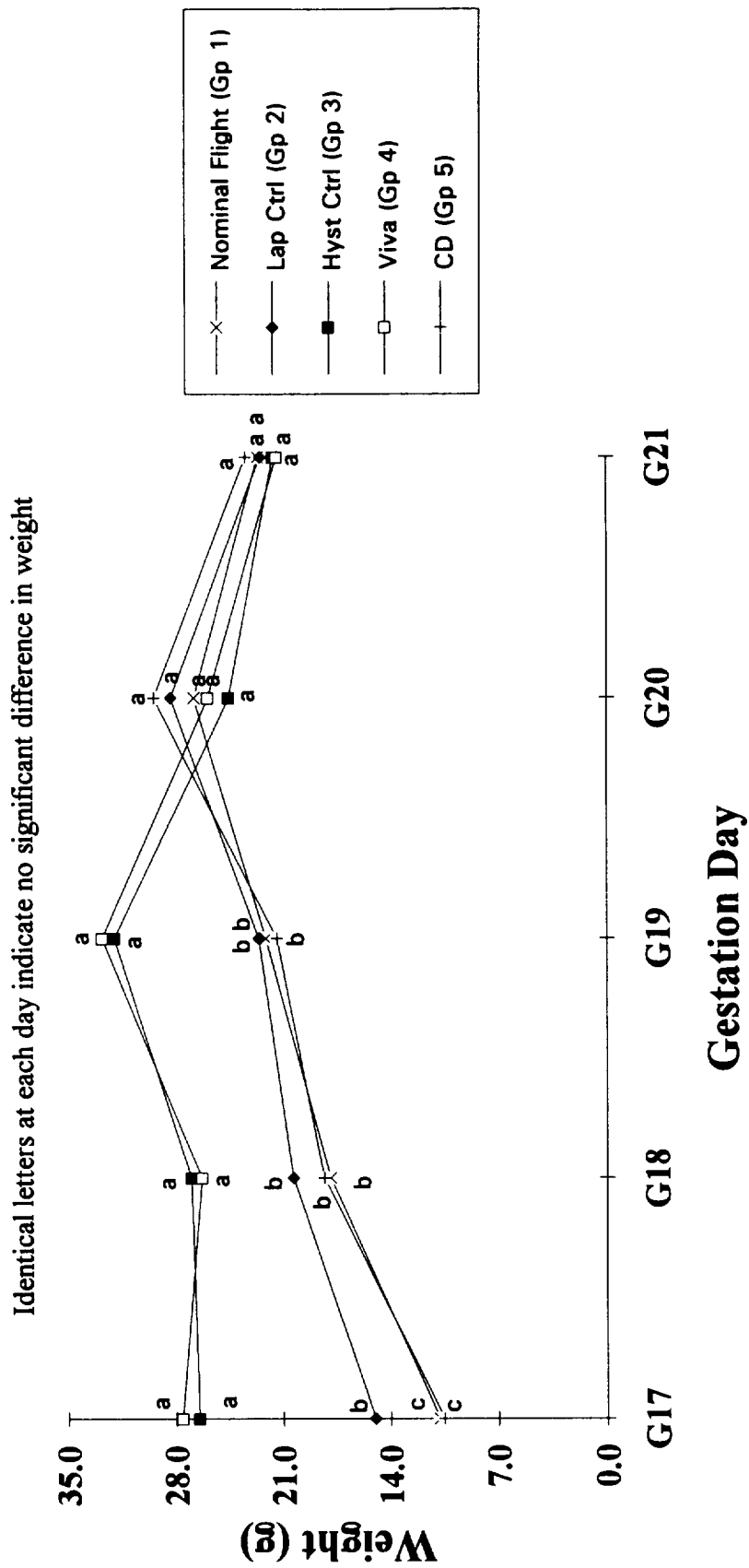


FIG.34 WATER CONSUMED (Mean \pm SEM) BY PREGNANT RATS G17 - G21

Identical letters at each day indicate no significant difference in weight

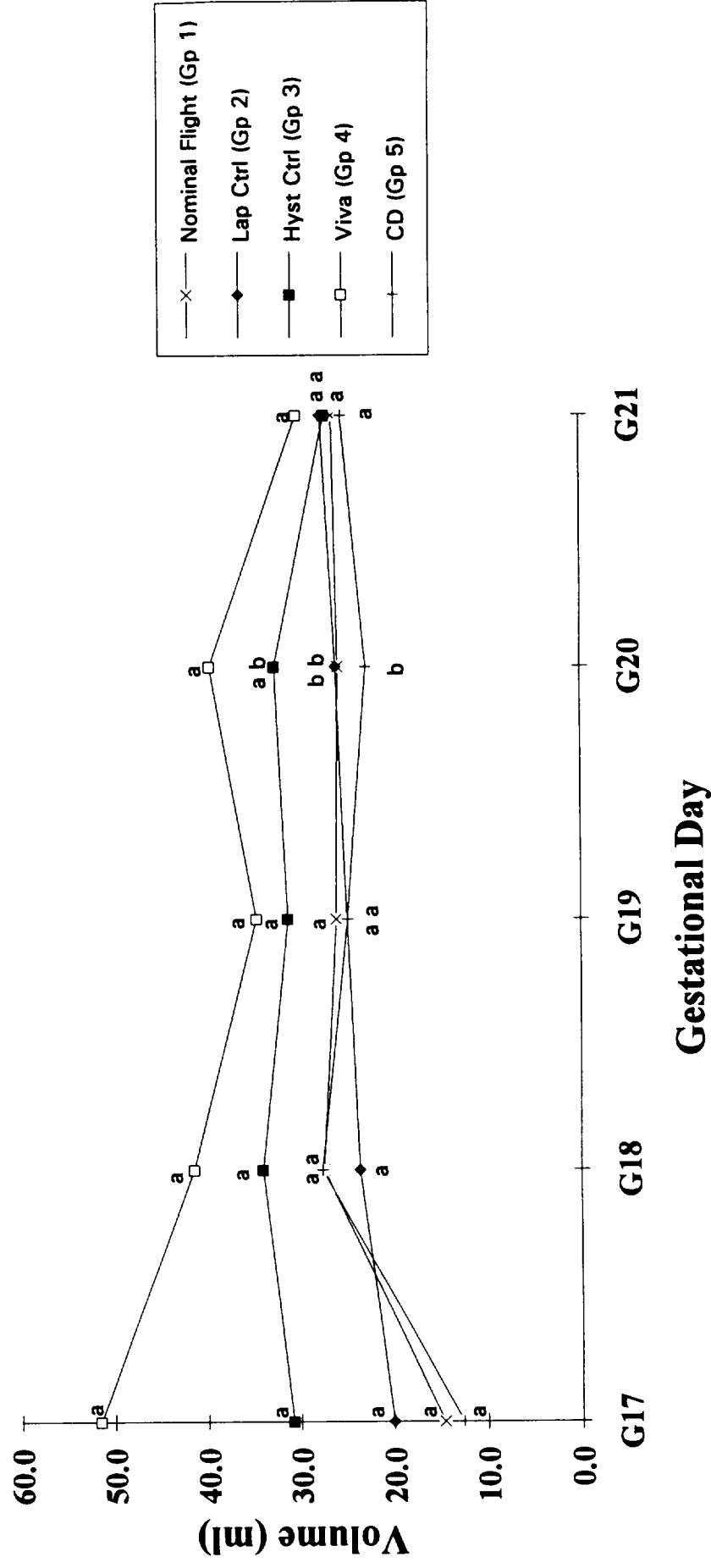


FIG.35 INITIATION OF DELIVERY: EXPERIMENTAL GROUPS

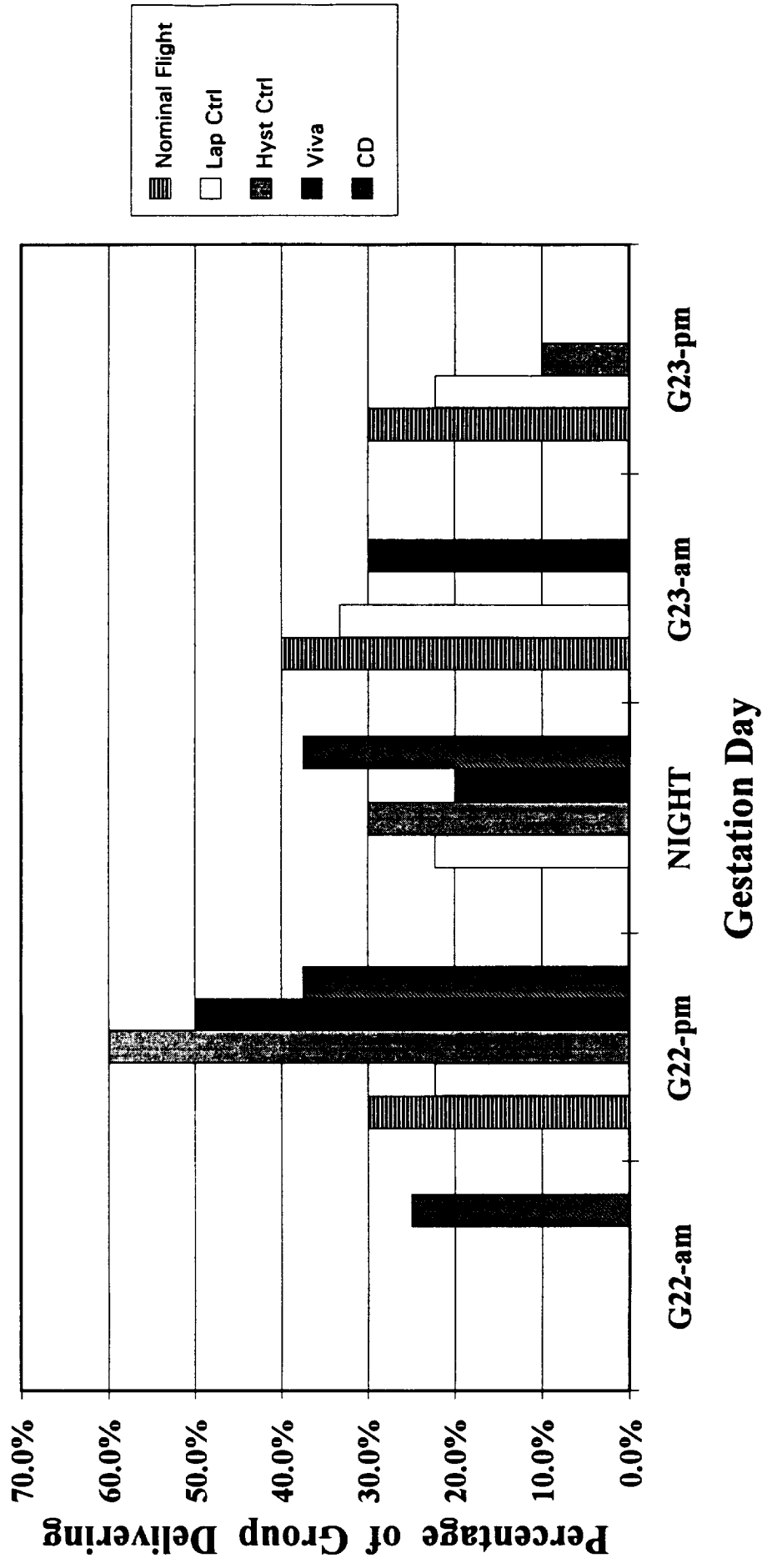
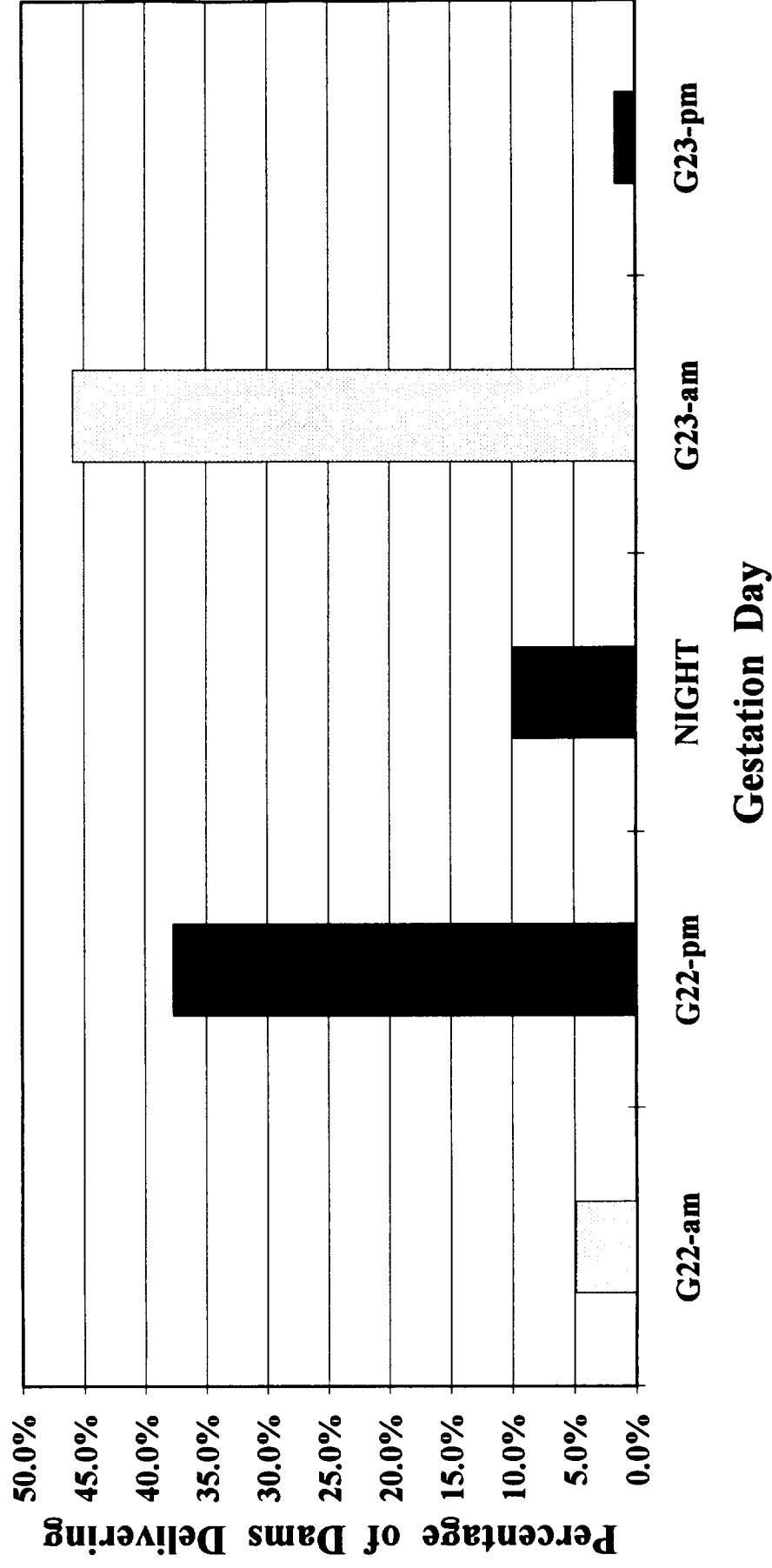


FIG.36 INITIATION OF DELIVERY: FOSTER DAMS



**FIG.37 NUMBER OF LIVE FETUSES (Mean +/- SEM) AT
G17 COMPARED TO NUMBER OF LIVE FETUSES
DELIVERED**

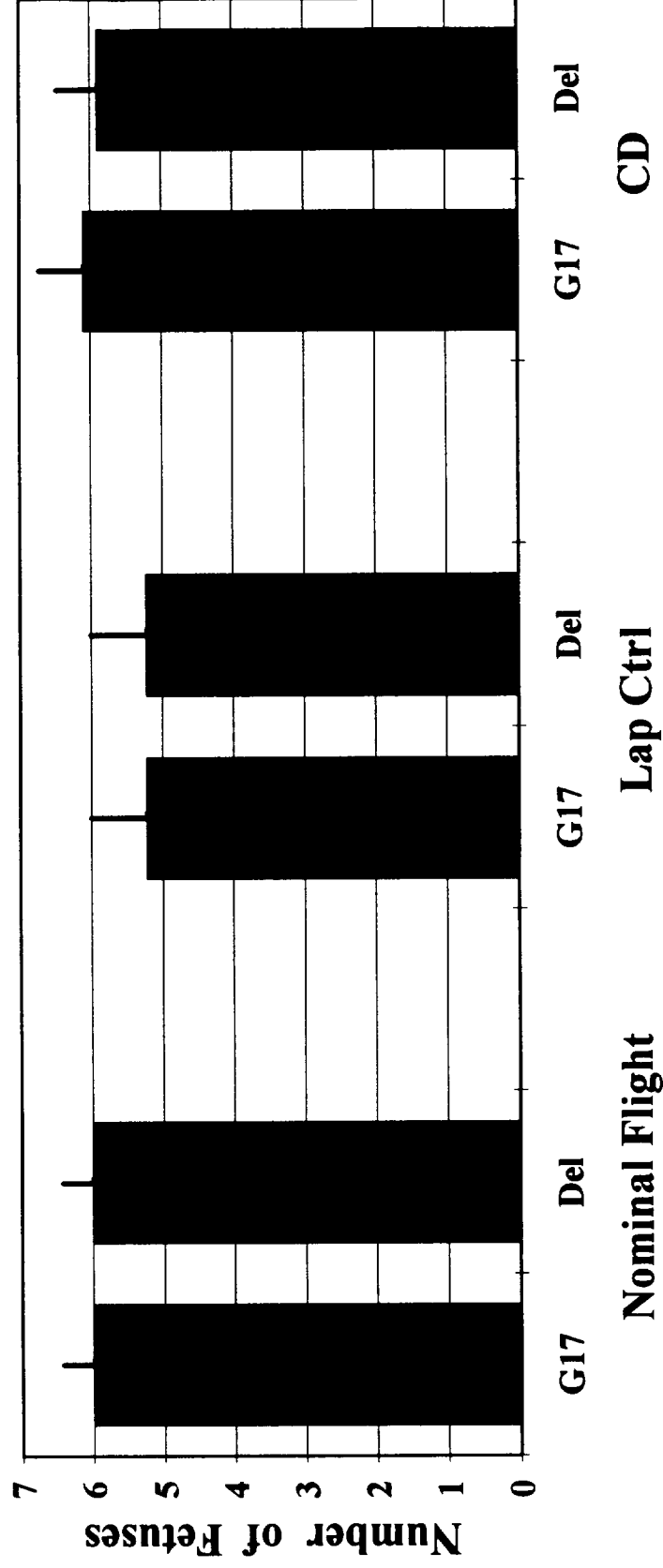
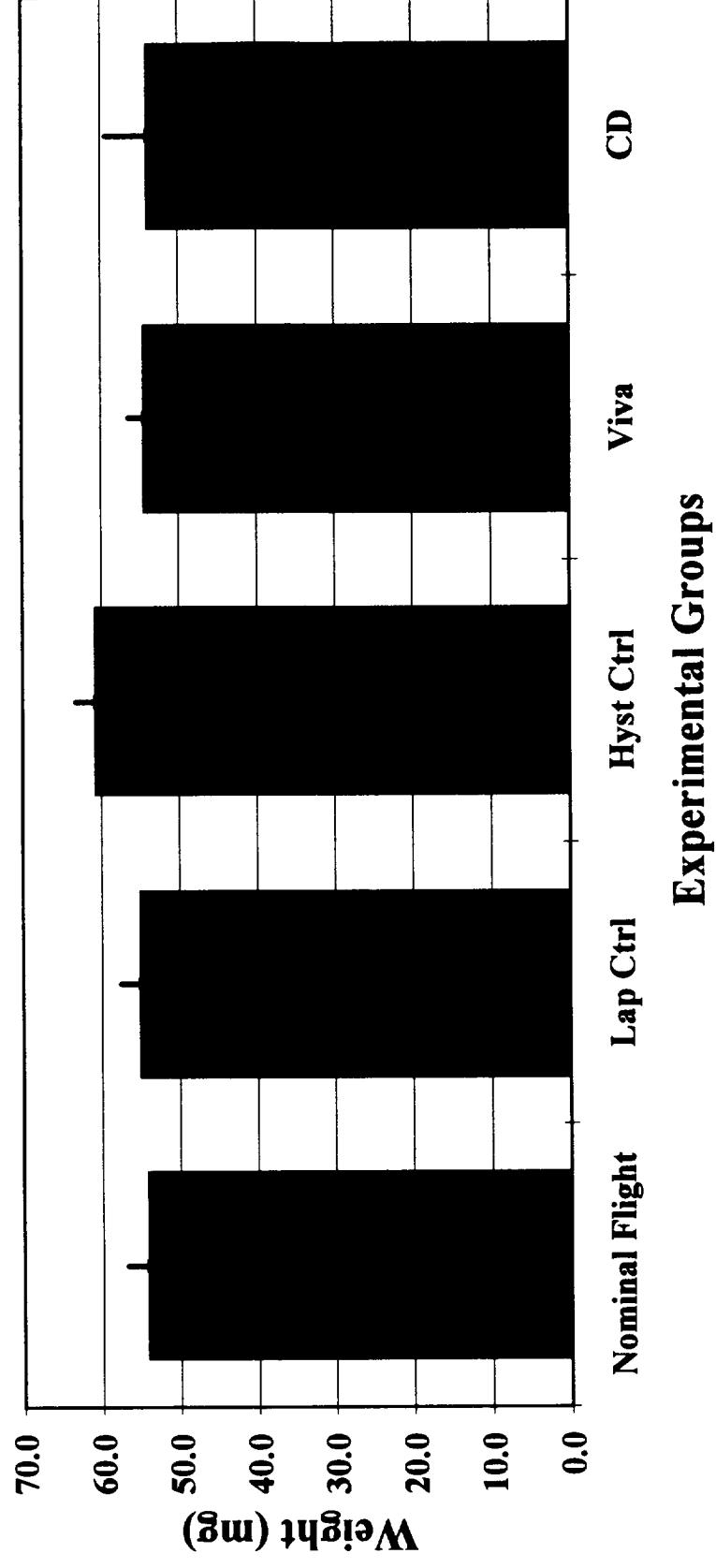
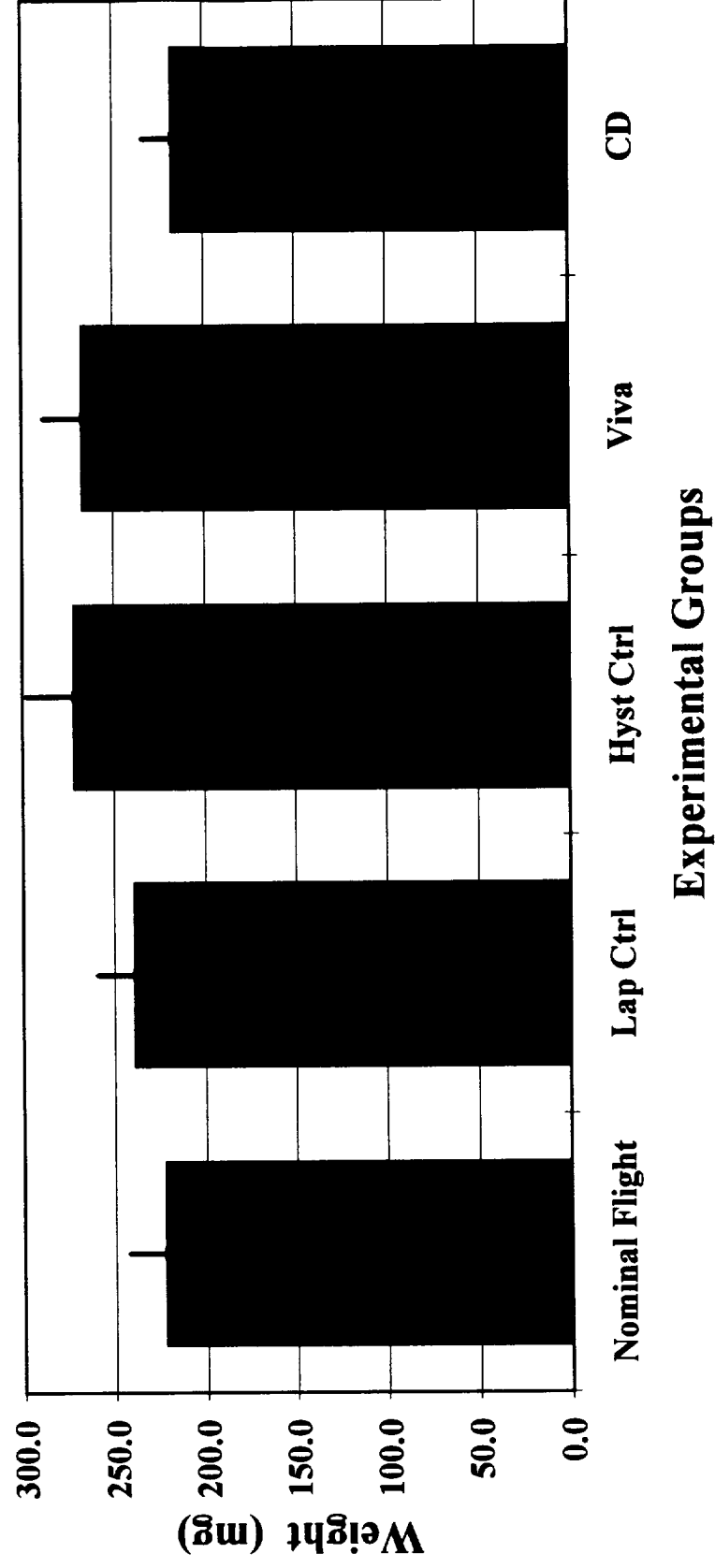


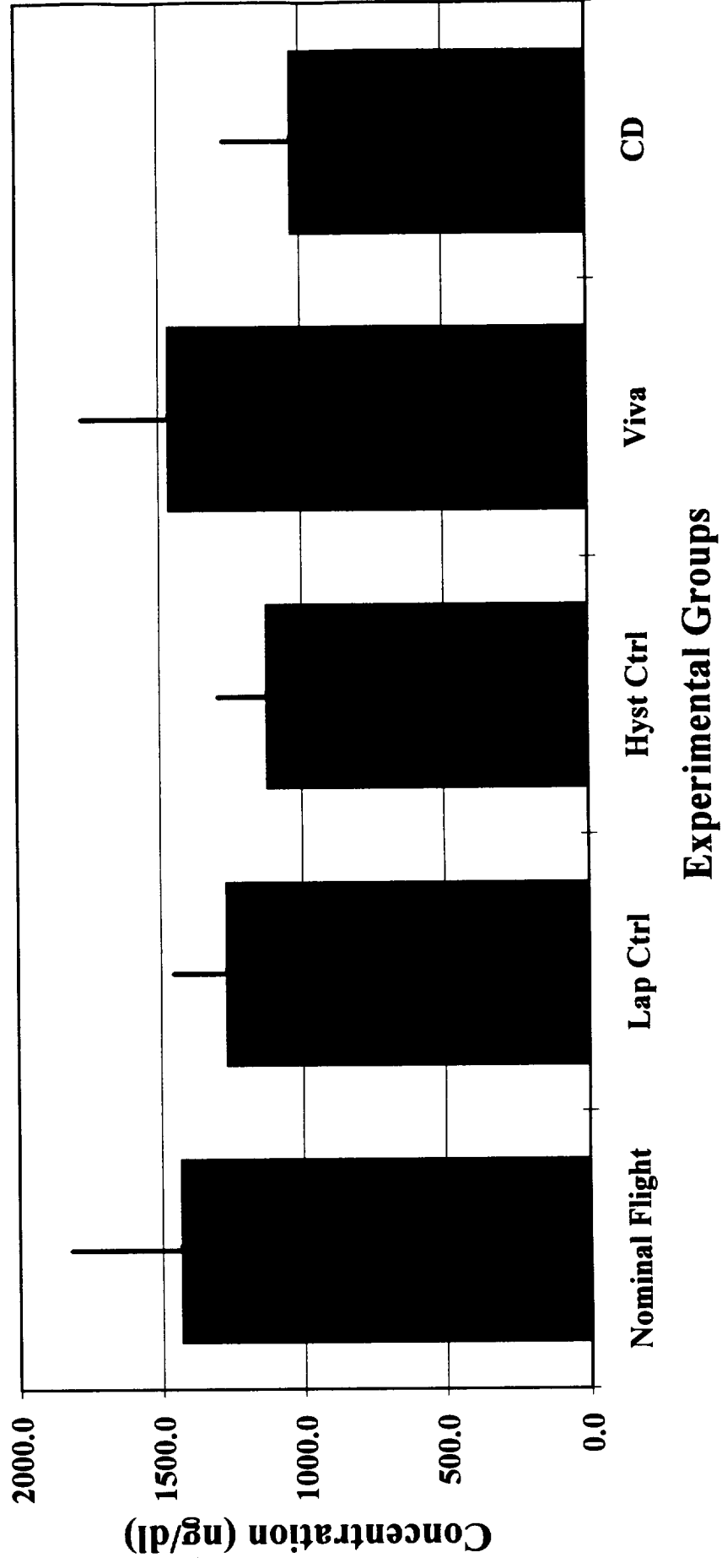
FIG.38 COMBINED ADRENAL WEIGHT (Mean +/- SEM)
OF DAMS AT DAY OF PARTURITION



**FIG.39 THYMUS WEIGHT (Mean +/- SEM) OF DAMS AT
DAY OF PARTURITION**



**FIG.40 PLASMA PROGESTERONE LEVELS (Mean +/- SEM) IN
POST-PARTUM DAMS**



**FIG.41 PLASMA ESTRADIOL LEVELS (Mean +/- SEM) IN
POST-PARTUM DAMS**

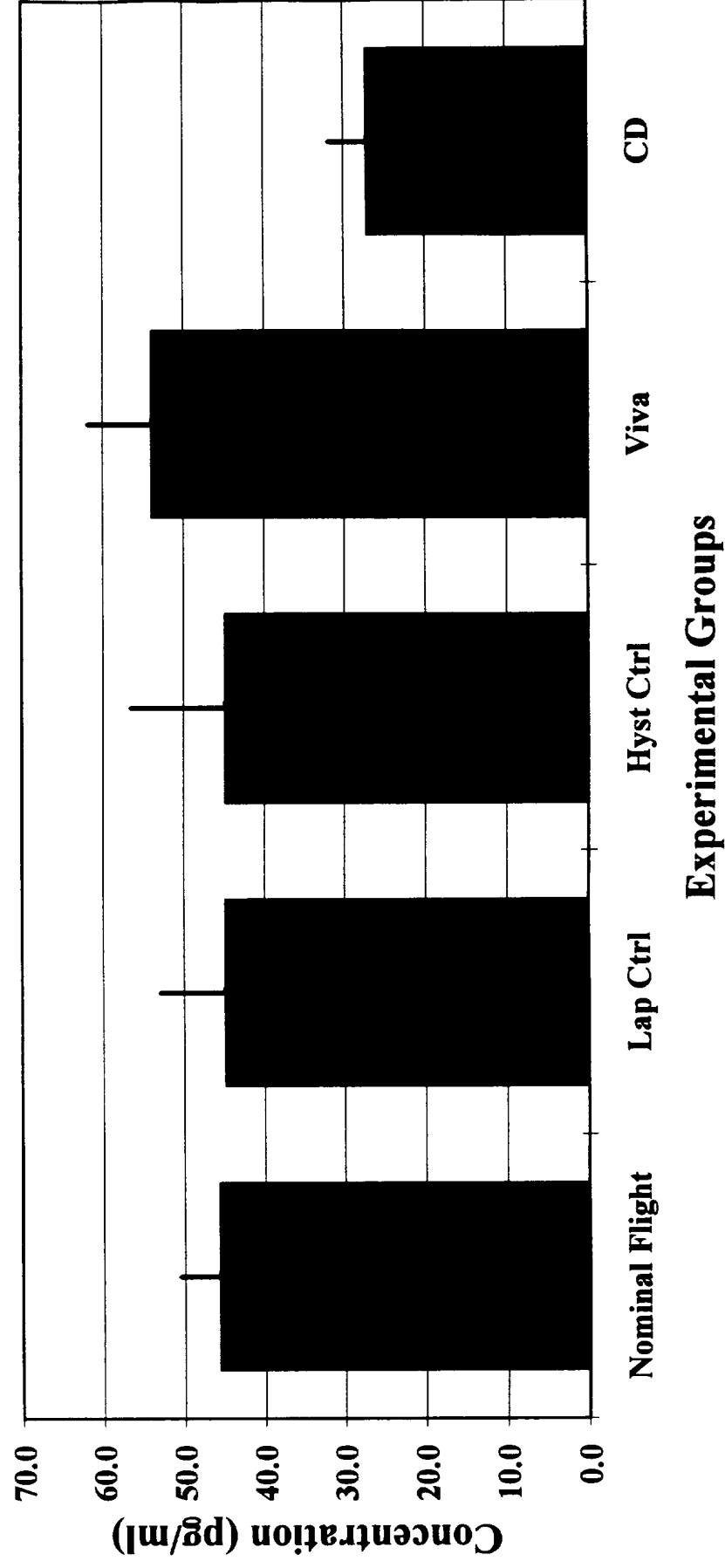


FIG.42 PLASMA CORTICOSTERONE LEVELS (Mean +/- SEM)
IN POST-PARTUM DAMS

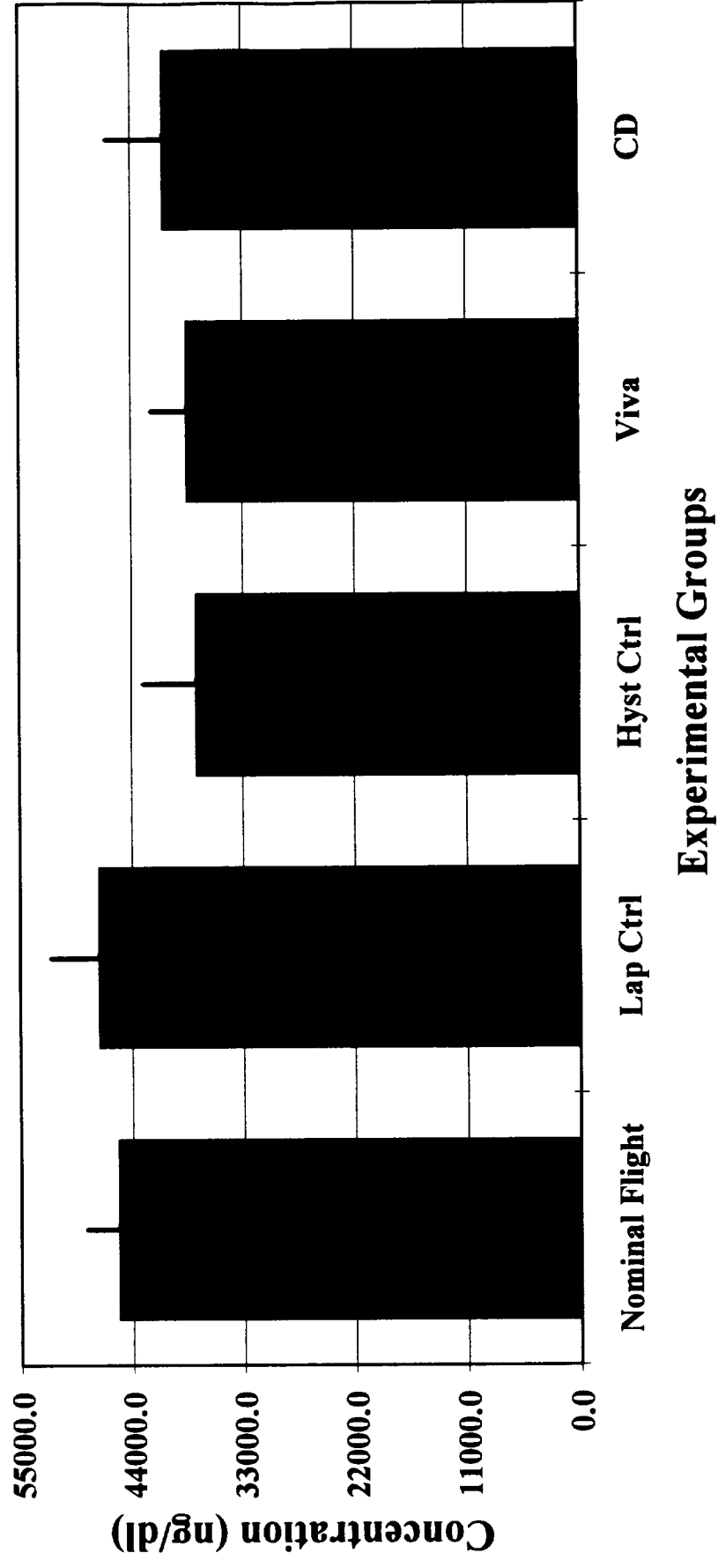
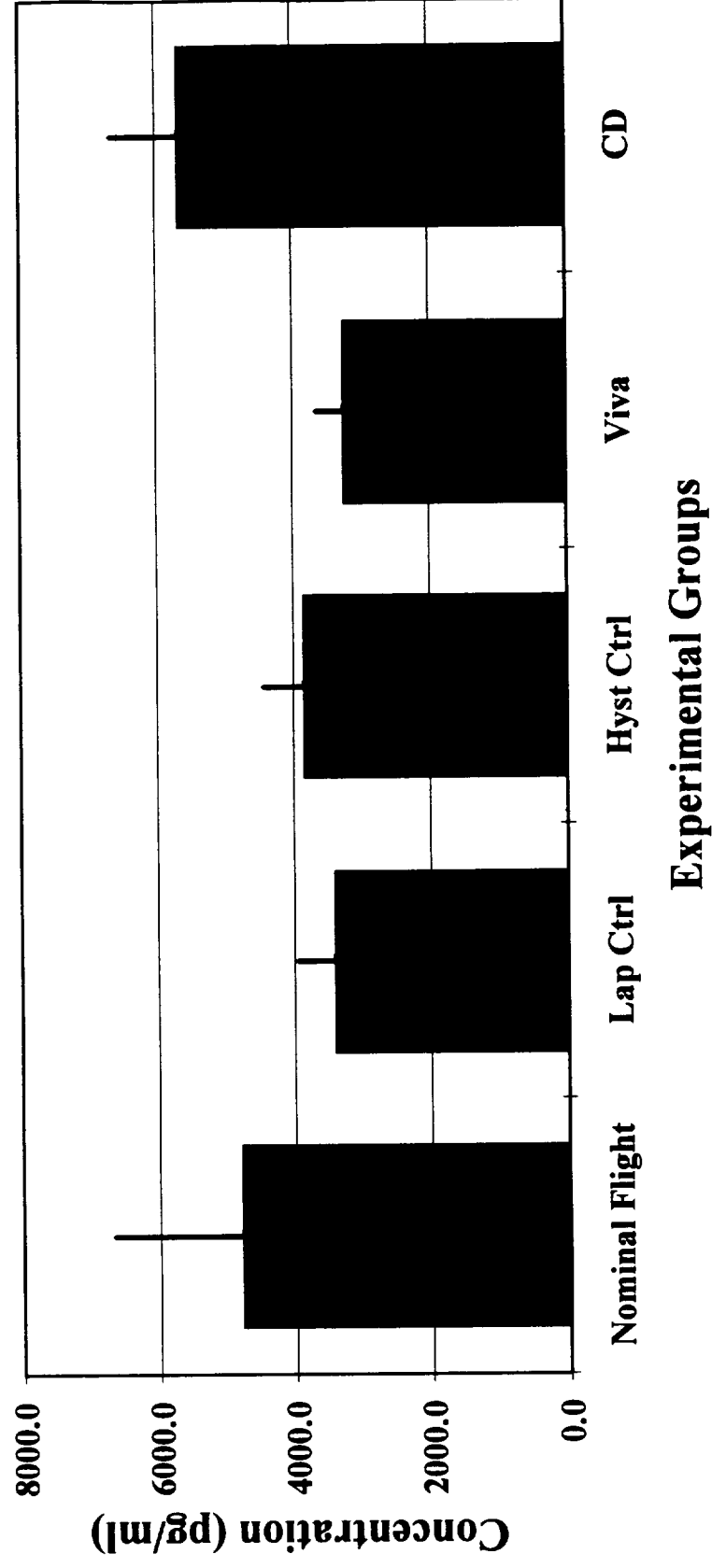


FIG.43 TOTAL PLASMA CATECHOLAMINE LEVELS
(Mean +/- SEM) IN POST - PARTUM DAMS



**FIG.44 PLASMA DOPAMINE LEVELS (Mean +/- SEM) IN
POST - PARTUM DAMS**

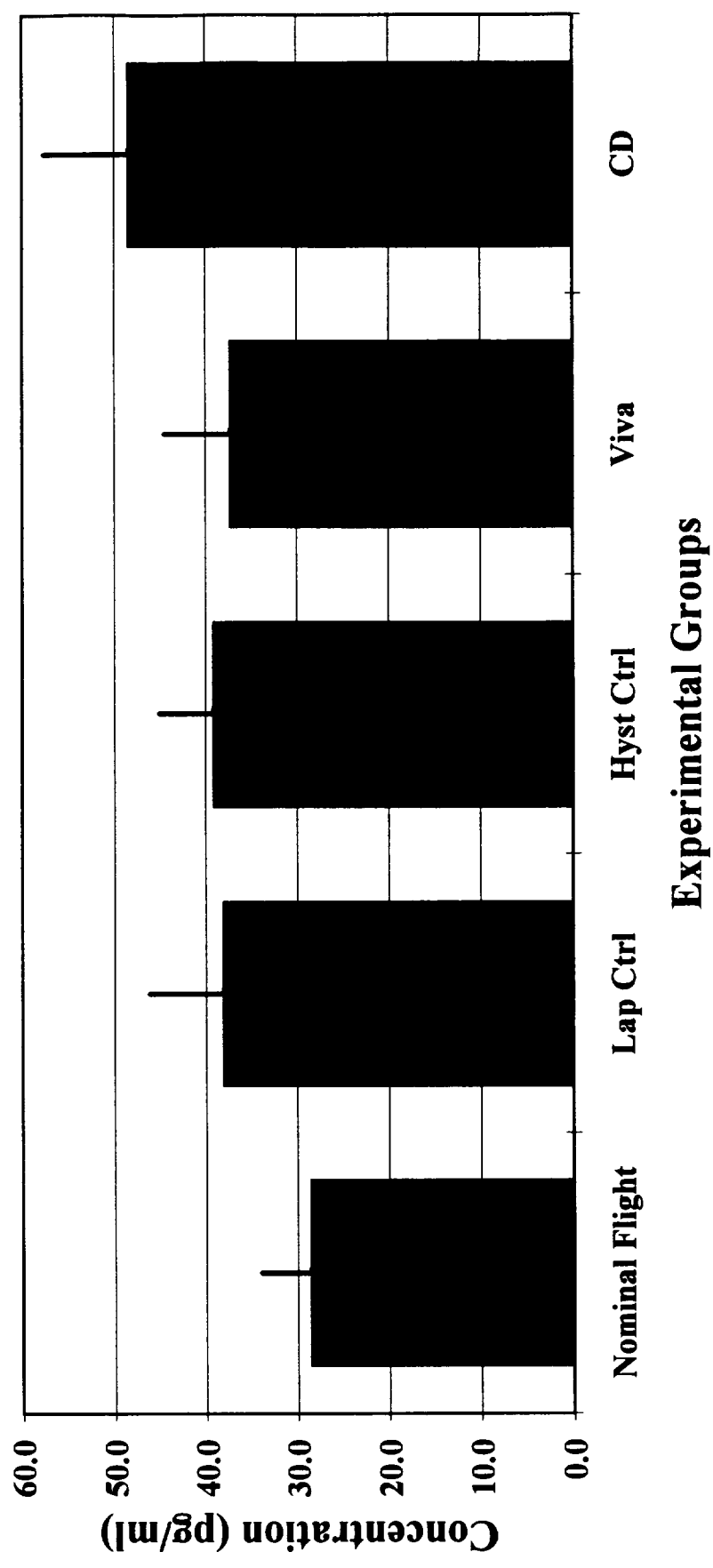


FIG.45 PLASMA NOREPINEPHRINE LEVELS (Mean +/- SEM)
IN POST - PARTUM DAMS

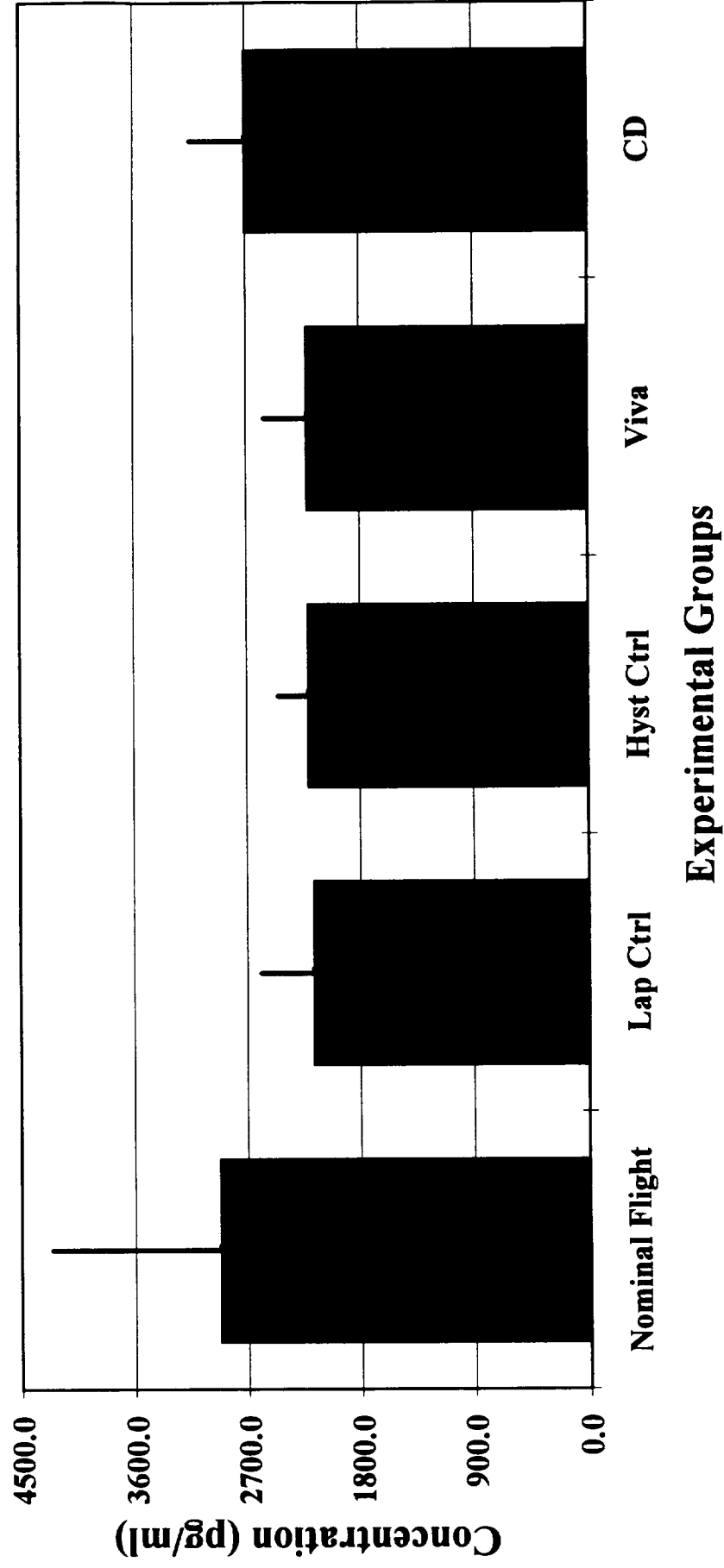
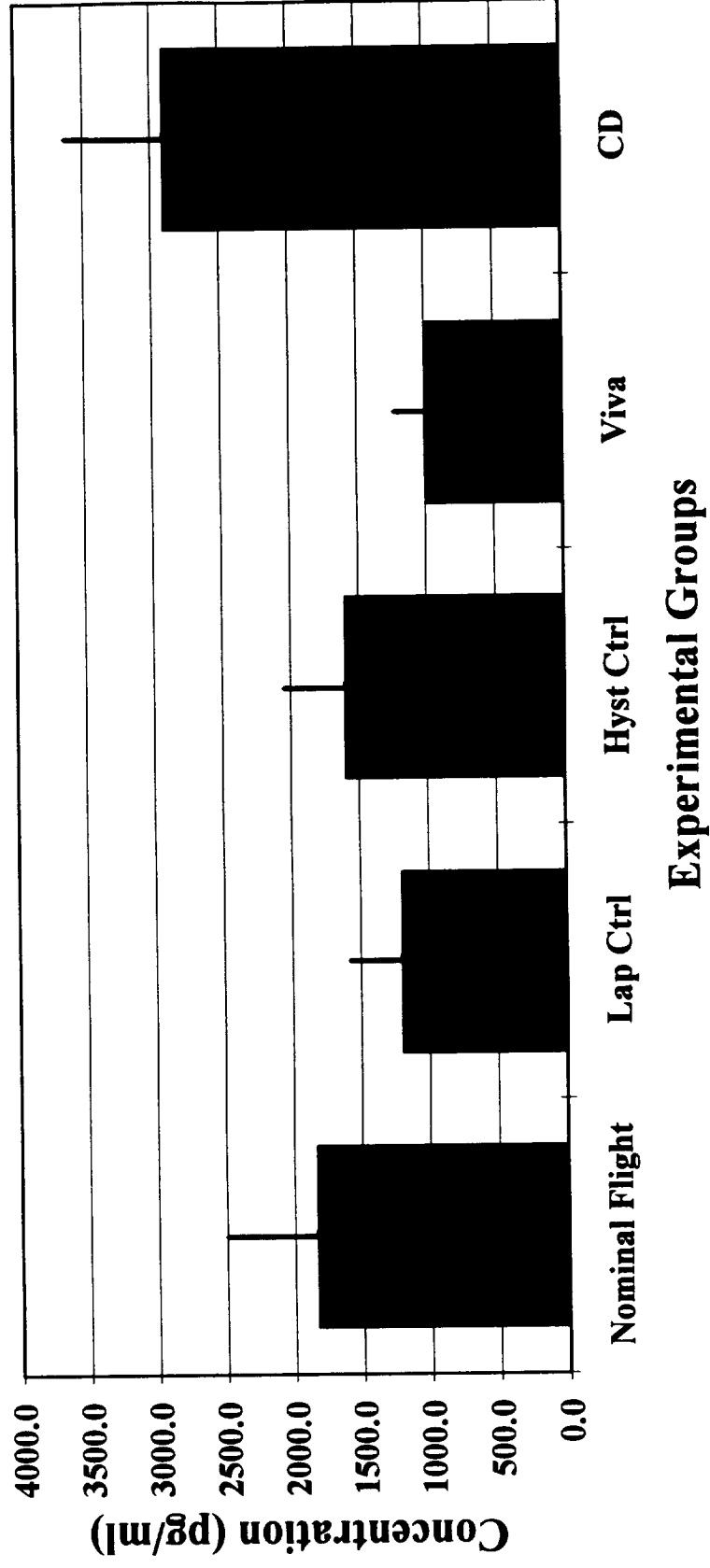


FIG.46 PLASMA EPINEPHRINE LEVELS (Mean +/- SEM)
IN POST-PARTUM DAMS



**FIG.47 ANOGENITAL DISTANCE (Mean +/- SEM) OF
MALE PUPS ON DAY OF DELIVERY**

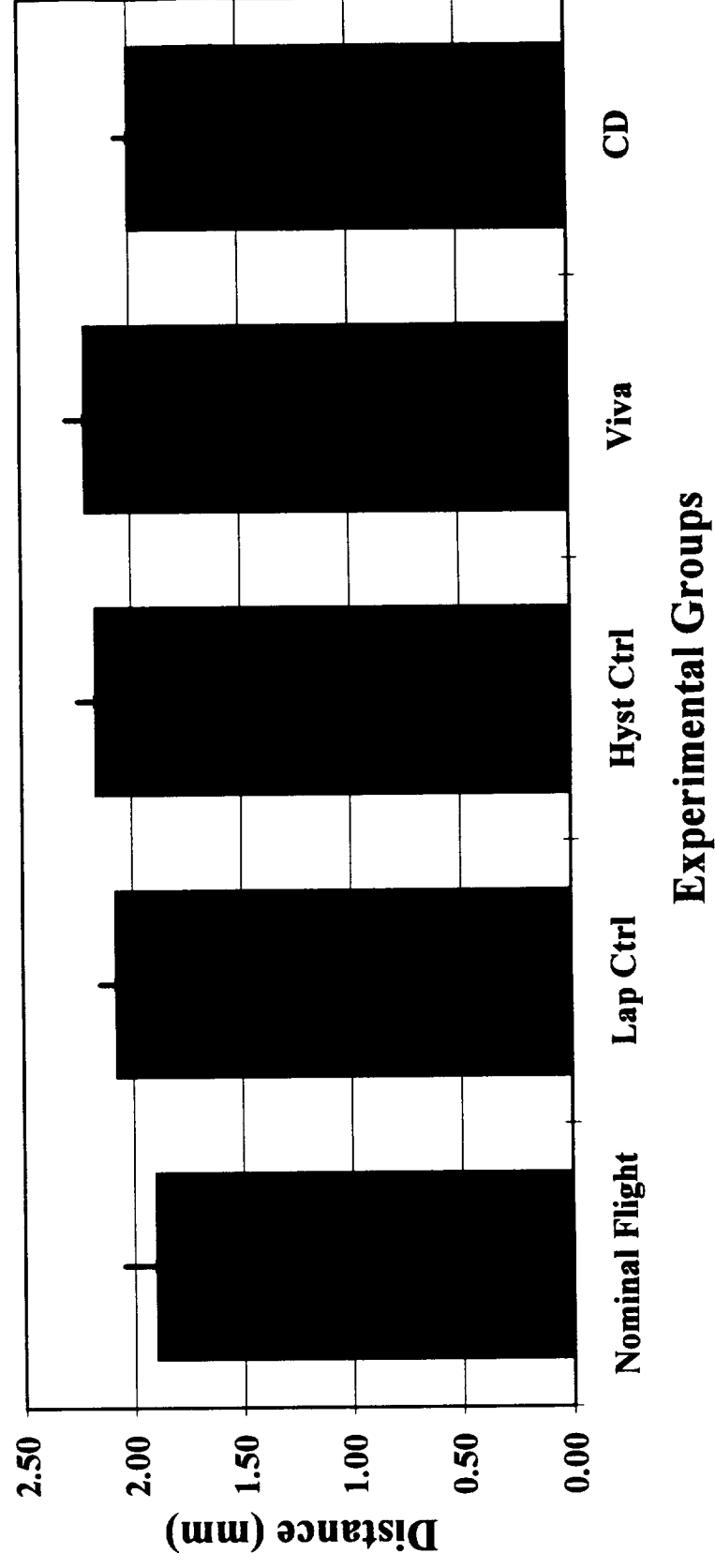
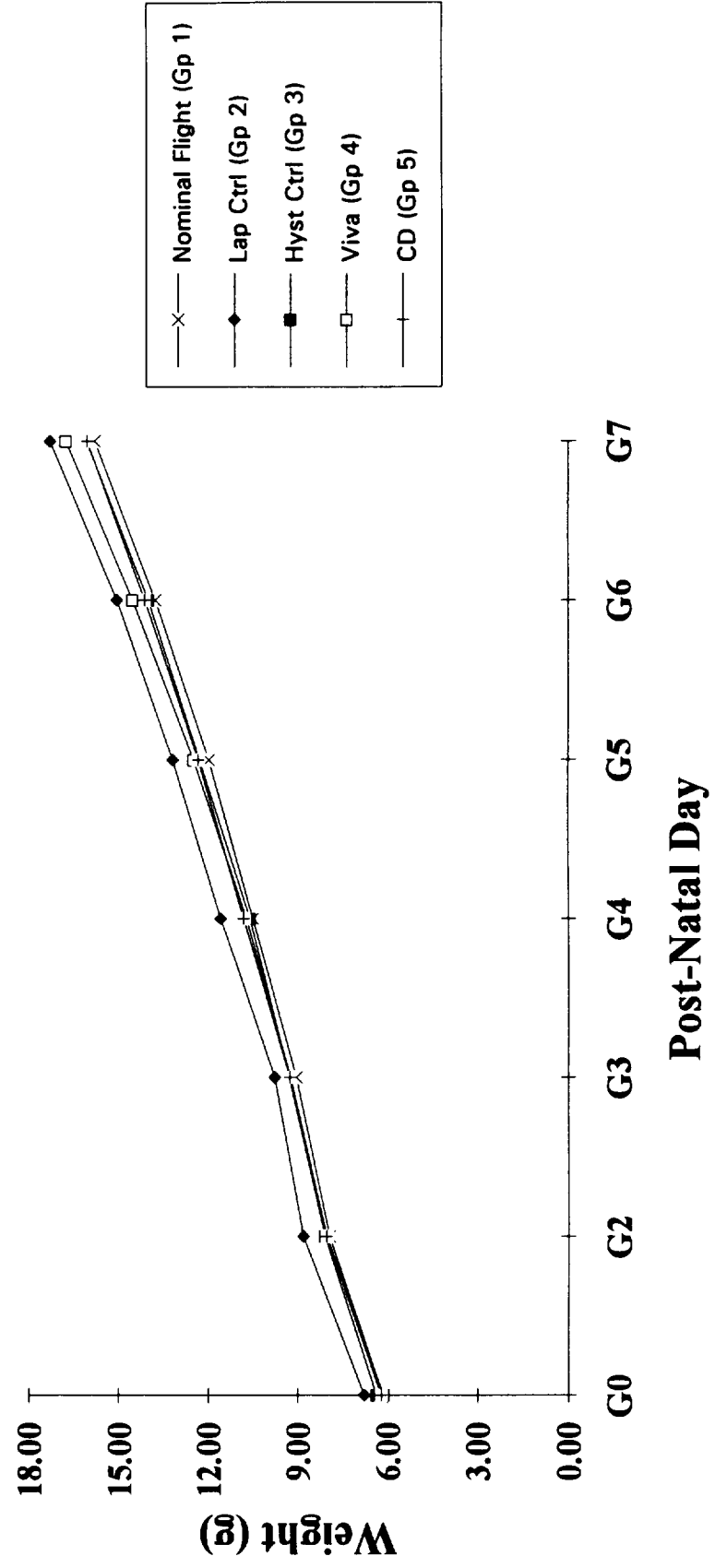


FIG.48 BODY WEIGHT (Mean +/- SEM) OF RAT PUPS
NEONATAL DAY 0 - DAY 7



**FIG.49 THYMUS WEIGHT (Mean \pm SEM) OF DAY 7
PUPS**

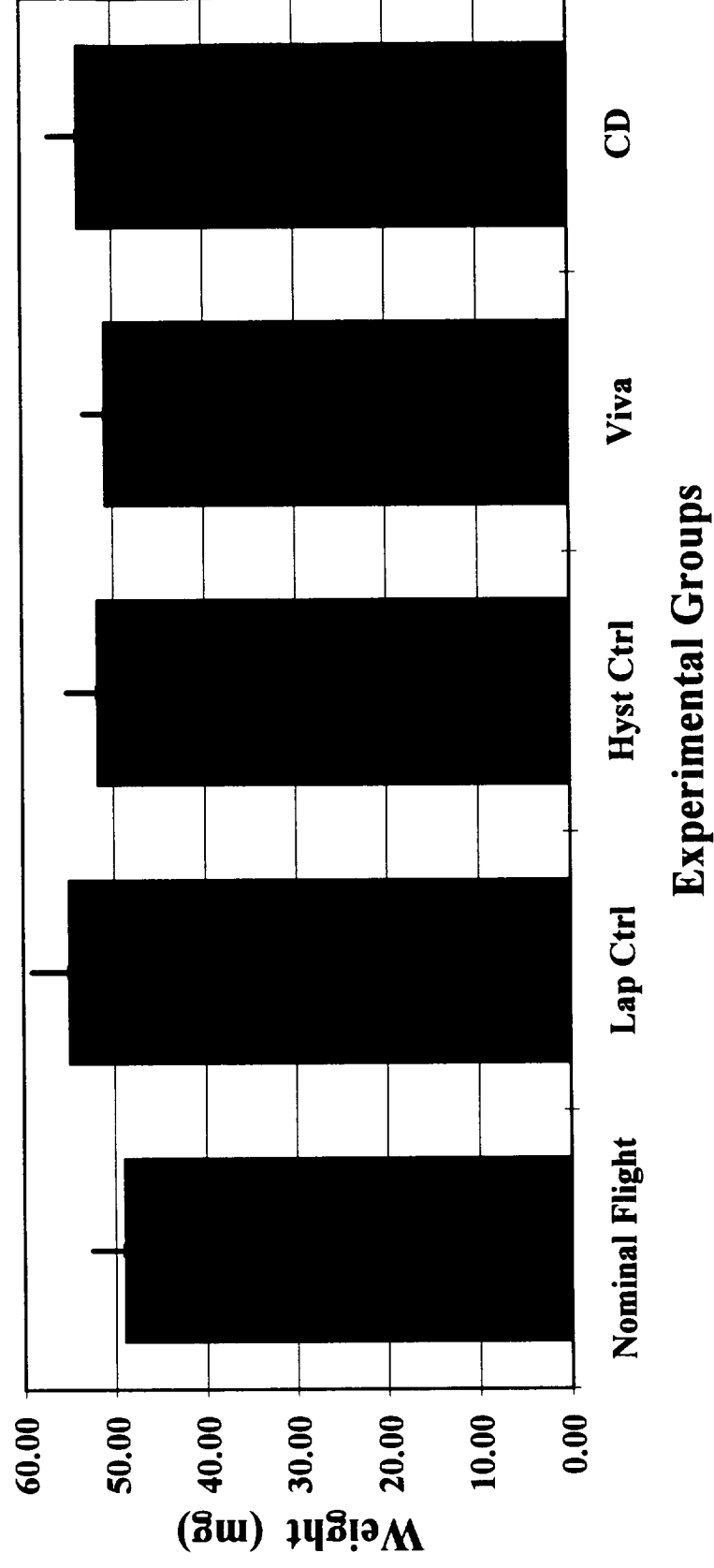


FIG.50 COMBINED ADRENAL WEIGHTS (Mean +/- SEM)
OF DAY 7 PUPS

